Vol. XII MARCH, 1927 No. 6

The Journal of Laboratory and Clinical Medicine

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VOL. XII ST. LOUIS, MO., MARCH, 1927 NO. 6

CLINICAL AND EXPERIMENTAL

THE VAN DEN BERGH REACTION FOR SERUM BILIRUBIN WITH NOTES ON INTERPRETATION AND TECHNIC*

BY W. W. HALL, M.D., WASHINGTON, D. C.

LIVER function tests are of late coming rapidly to the fore. The work done by the physiologist in establishing methods for the study and evaluation of the normal and the work of the clinical pathologist have combined to give us a number of tests by means of which the various functions of the liver may be studied.

Some of the proposed procedures seem to have very little foundation when studied by experimental physiology.[1] This may be because disease affects the liver function in a different way than simple removal of increasing amounts of hepatic tissue; disease may cause a qualitative as well as a quantitative deviation from the normal; the physiology of the human liver may differ from that of the experimental animal or because the test used is an "index of disease not necessarily wholly hepatic."[1]

The van den Bergh appears to be one of the liver function tests for which the least that may be said is that it serves to measure degree and differentiate type of bilirubinemia in the causation of which the liver may be directly or indirectly but not necessarily alone at fault. Van den Bergh developed his reaction by applying the Ehrlich diazo reaction to sera containing bilirubin. The reaction depends upon the formation of azobilirubin, a red dye, when an acid solution of a diazonium salt is added to bilirubin in solution. Van den Bergh found that pure bilirubin in a dilution of 0.7 mg. per liter gave a positive reaction and that allied substances such as biliverdin did not.

Two types of jaundice are differentiated by the van den Bergh reaction and their differentiation depends upon the fact that bilirubin in one type (obstructive) combines promptly with the diazo reagent to form azobili-

*From the Laboratories of the U. S. Naval Medical School, Washington, D. C.
Received for publication, November 18, 1926.

rubin (direct reaction), while in the other (hemolytic) type it seems to be bound in such a way that the reaction is long delayed or negative, but if alcohol be added the reaction occurs promptly (indirect reaction). The two general groups of jaundice, obstructive and hemolytic, are caused by pathologic lesions of distinctly different type and location.

A résumé of the theory of bilirubin formation and excretion may be of help in the discussion of van den Bergh's classification. Erythrocytes are constantly being destroyed in the body and bilirubin is formed from the liberated hemoglobin by cells of the reticuloendothelial system. These cells are found throughout the body in the endothelium of vessels and capillaries but most abundantly in the sinusoids of the spleen lymph glands and liver.

The bilirubin thus formed is presumed to be present in combination in the blood stream. As the blood passes through the liver the parenchymatous or polygonal cells extract the bilirubin and excrete it into the bile canaliculi. When for any reason the flow of bile is obstructed this pigment (with the bile salts) passes again into the blood by absorption. In this type of jaundice the bilirubin combines directly with the diazo reagent, upon its addition, to form the red azo dye. That pigment, present in small but constant quantity normally and in much larger amounts in conditions characterized by increased destruction of red cells, reacts only in the presence of alcohol which is thought to split it from its protein complex. Van den Bergh calls the former type of pigment obstructive and the jaundice in those conditions mechanical, while the jaundice of hemolysis he calls dynamic.

S. M. Rosenthal[2] says that in attempting to discover the mechanism by which certain dye stuffs and bilirubin are excreted by the liver he has studied their behavior from a physicochemical standpoint. By ultrafiltration experiments he has determined that they circulate firmly bound to the serum proteins. This prevents their elimination by the kidneys. Bile salts by their effect on surface tension are able to liberate bilirubin and these dye stuffs from their adsorption compound with the protein so that they can be further excreted by the liver.

Van den Bergh and his followers originally considered bilirubin in the two types of jaundice to be essentially different, the bilirubin which passed the polygonal cells of the liver having undergone some fundamental change as it thereafter reacted as the bilirubin in the bile itself. The essential difference is apparently not in the bilirubin itself but rather in the presence or absence of substances which act as do the bile salts to split the bilirubin from its protein complex and allow prompt union with the diazo reagent.

The rôle of the liver in bile pigment metabolism has recently been quite definitly established by Mann's[3] experiments. He has proved that following total removal of the liver bilirubin rapidly accumulates in the blood, thus establishing the fact that the liver acts principally, with reference to bilirubin, as an excretory organ and that the transformation of hemoglobin to bilirubin is carried on efficiently quite independently of the parenchymal cells of that organ, presumably by the reticuloendothelial cells throughout the body.

McNee (as quoted by Bockus and Shay[4]) classifies jaundice as (1) obstructive hepatic, (2) toxic and infective, (3) hemolytic, and cites the biphasic

reaction (discussed under interpretation) as characteristic of the toxic and infective group. The reactions with blood from cases belonging clinically to this group have been very variable, some being prompt direct, some delayed and others negative direct. Recent work indicates[5] that bile salts are synthesized as well as excreted solely by the liver and that the amount of bile salts formed may be diminished in some cases of liver malfunction. In view of the probable rôle of bile salts in the van den Bergh (qualitative) this may prove of importance in the interpretaion of the reaction and help to explain some apparent contradictions in the toxic and infective group as well as with blood from patients in the early stages of obstructive jaundice.

Technic of the Method.—Draw 5 c.c. of blood by venipuncture into a dry centrifuge tube and allow to clot. Separate serum by centrifuge if necessary, and pipette off. The diazo reagent which must be made up just before use is a mixture of two solutions:

*Solution A.**	
Sulphanilic acid	1.0 gm.
Conc. HCl	15.0 c.c.
Distilled water qs.	1000.0 c.c.
Solution B.	
Sodium nitrite	0.5 gm.
Distilled water	100.0 c.c.
To prepare fresh reagent.	
Solution A.	25.00 c.c.
Solution B.	0.75 c.c.

*Reagents must be of best quality. Sulphanilic acid must be reasonably fresh. Very old samples have failed to react well.

*The Qualitative or Direct Reaction.**—Place 0.25 c.c. serum in each of three small test tubes. To tube No. 1 add 0.2 c.c. water. To tube No. 3 add 0.2 c.c. diazo reagent (fresh). After waiting five minutes for reaction to become complete in control tube No. 3, add 0.2 c.c. diazo reagent to tube No. 2. Watch and time development of any reaction. Prompt or immediate reaction begins before thirty seconds have elapsed. Comparison with serum control, tube No. 1, and completed reaction control, tube No. 3, will aid in detection of color.

The Quantitative Test or Indirect Reaction.†—To 1 c.c. serum in a 15 c.c. graduated centrifuge tube add 0.5 c.c. diazo reagent (freshly prepared as above). After a minute or two add 2.5 c.c. 95 per cent alcohol and 1.0 c.c. saturated solution of ammonium sulphate $(NH_4)_2SO_4$. Mix well with a stirring rod after each addition and finally centrifuge.

The diazo reagent is added before the alcohol to allow "coupling"[6] to take place. By this method very little if any bilirubin is carried down with the precipitated protein, as the azobilirubin is very soluble in alcohol while bilirubin is less so and is carried down with the precipitate in relatively large amounts if the reagents are added in the reverse order. The color of the supernatant fluid will vary from a faint pink color as in normal serum, to a deep violet, depending on the amount of bilirubin present. (Chylous sera give cloudy solutions which are objectionable for colorimetric comparisons.)

*From note on improvement in technic.[6] We have, however, discarded the use of caffeine sodium salicylate, for we have found that its effect was very inconstant as did McNee and Keefer.[6]

†Quantitative method modified from Ravdin.[7]

The quantity of supernatant fluid is read on the graduations of the centrifuge tube (Fig. 1) and the dilution of the bilirubin contained in the cubic centimeter of serum used is thus directly obtained. The quantity of bilirubin present in the serum (1 c.c.) is now, as azobilirubin, entirely in alcoholic solution. This supernatant alcohol usually varies from 2.5 to 3.0 c.c. The calculation of the dilution (1 in 4) as used by Ravdin[7] and others does not appear to be accurate. We have had no difficulty in reading the amount of supernatant alcoholic solution, as the ammonium sulphate, protein and alcohol layers separate very sharply on centrifuging. (Fig. 1.) As the color of the standard represents a bilirubin concentration of 5 mg. per liter, the calculation is:

$\frac{\text{Standard}}{\text{Unknown}}$ × Dilution of Unknown * × 5 = mg. bilirubin per liter of serum, (using a plunger type of colorimeter).

$\frac{\text{Unknown}}{\text{Standard}}$ × Dilution of Unknown * × 5 = mg. bilirubin per liter of serum, (using a dilution type of colorimeter).

Standard for the quantitative reaction:*

Solution 1.		
Ammonium ferric alum	0.1508	gm.
Conc. HCl	50.0	c.c.
Distilled water qs.	100.0	c.c.
(Keeps indefinitely.)		
Solution 2.		
Of solution No. 1	10.0	c.c.
Conc. HCl	25.0	c.c.
Distilled water q.s.	250.0	c.c.
(Keeps about one month.)		
Standard which is made fresh daily.		
Of solution No. 2	3.0	c.c.
10% ammonium sulphocyanate or		
20% potassium sulphocyanate	3.0	c.c.
Ether	12.0	c.c.

*As read from the supernatant alcoholic solution in graduated centrifuge tube.

Shake thoroughly. The ether extracts the color from the solution and forms a supernatant layer which may be used in colorimetric comparison. The standard matches in color a dilution of 5 mg. per liter of bilirubin.

By the use of cobaltous sulphate as suggested by van den Bergh and first published by McNee and Keefer[6] a permanent aqueous standard may be made which avoids many of the errors and difficulties inherent in the ether standard. They advise the use of 2.161 gm. anhydrous cobaltous sulphate to 100 c.c. water. This standard also represents the color given by 5 mg. bilirubin per liter. We, however, found it difficult to obtain or make anhydrous ($CoSO_4$) cobalt sulphate as decomposition took place while the water of crystallization was being driven off. It is also impossible to obtain an accurate weight using the crystalline salt, allowing for the seven molecules of water of crystallization ($CoSO_4.7H_2O$), as the salt is somewhat efflorescent and perfect crystals almost never are found. We, therefore, suggest that the cobalt sulphate standard be made up as follows: Make an aqueous solution somewhat deeper in color than the ether standard; compare in colorimeter and dilute as indicated to match the color in the ether standard. This solution keeps well in the dark. We have found that the addition of 0.5 c.c.

H_2SO_4 per 100 c.c. does not change the color and the solution keeps thus indefinitely.

Interpretation.—The normal range of bilirubin as given by McNee,[6] Ravdin[7] and others is from 1 to 3 mg. per liter, latent jaundice from 4 to 20 and clinical icterus from 20 up. The dividing line between latent and clinical icterus should probably be one or two points lower. We have seen some cases which were clinically mildly jaundical with only 18 mg. bilirubin per liter serum. If reported in units, one unit equals 5 mg. bilirubin per liter.

There are three possible results in the direct reaction:

Immediate or prompt, beginning before thirty seconds have elapsed and reaching its maximum in about two minutes.

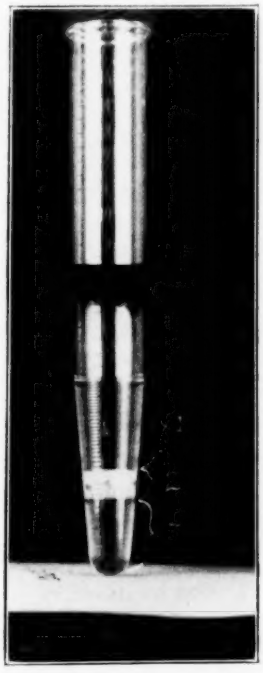


Fig. 1.—Quantitative van den Bergh reaction completed. Note sharply separated layers. (1) Supernatant alcoholic layer containing in solution as azobilirubin all the bilirubin present in the serum added (1 c.c.). (2) Layer of precipitated proteins. (3) Ammonium sulphate layer. The supernatant fluid varies in each test according to evaporation and fluid interchange but averages about 3 c.c. The dilution of bilirubin is therefore 1 in 3 (approximately). The exact amount must be read in each case and that figure used.

Delayed, beginning after thirty seconds. These reactions develop slowly. The longest in our experience has been thirty minutes, although McNee and Keefer[6] report delayed reactions which took one hour to develop.

Negative, no color developed in thirty minutes.

The prompt direct reaction is given by the bilirubin in the obstructive type of jaundice. Delayed or negative reactions may be obtained in both normal sera and those from cases of nonobstructive or hemolytic jaundice. We have dropped the term "biphasic" reaction which was used to describe a reaction beginning promptly and not reaching its maximum until after thirty seconds, since we found as did Andrews[8] that no specimens, no matter how intense a

prompt direct reaction they gave, developed their maximum color before one to two minutes had elapsed. We have therefore classed as prompt direct any reaction beginning before thirty seconds. The delayed and negative direct reactions may be grouped together as both may be obtained in normals and in nonobstructive jaundice.

The direct reaction serves both to measure the bilirubin and to develop a color with bilirubin in the presence of alcohol which gave none in the direct reaction. Thus it demonstrates and measures the jaundice of hemolytic origin and brings to light a jaundice of latent type, that is, one in which the concentration of bilirubin has not reached the level at which it can be demonstrated in the urine by the usual tests nor can be detected clinically in the sclera and skin. We have found no case in which color was entirely absent in the quantitative or indirect reaction, although many, in fact most, normals gave a reading of less than 1 mg. per liter (0.2 unit). All types of bilirubin give color in the indirect reaction, so that to report a positive indirect reaction without reference being made to the type of direct reaction is to give incomplete information.

We do not agree with McNee and Keefer[6] that the reactions change on standing and that specimens which originally gave a prompt direct reaction give a long delayed reaction later. We have kept sera as long as three months and at the end of that time they still gave a prompt direct reaction as before.[9] Slight hemolysis has not interfered with the reactions.

SUMMARY AND CONCLUSIONS

1. The van den Bergh reaction is a means of qualitative (direct reaction) and quantitative (indirect reaction) study of serum bilirubin. The presence of bile salts in serum of obstructive jaundice probably explains the prompt direct reaction of the bilirubin in this type and is the essential difference in sera of obstructive and hemolytic jaundice.

2. The term biphasic was used to describe a direct reaction which began before thirty seconds had elapsed, following the addition of the diazo reagent, and increased in intensity for some time after the thirty second limit. This designation (biphasic) is misleading and should be dropped, for no sera, even those most heavily jaundiced (obstructive), reach their maximum color until long after thirty seconds have elapsed. We have therefore classed the direct reactions as follows: prompt or immediate, any reaction beginning in thirty seconds after addition of the reagent to the serum; delayed, a reaction beginning slowly after thirty seconds and reaching its maximum very slowly, sometimes taking thirty minutes or longer; negative, those in which no color at all developed.

3. In calculation of the dilution in the quantitative reaction the quantity of supernatant alcoholic solution should be read and that figure used (Fig. 1), as the bilirubin in the serum (1 c.c.) is all present as azobilirubin in the alcohol.

4. The aqueous cobalt sulphate standard is more desirable than the ether standard. It is more easily handled, is not subject to rapid evaporation and

can be made up in quantity. Comparison with the ferric-sulphocyanate-ether standard is recommended as a means of avoiding gravimetric errors, if a crystalline cobaltous sulphate is used ($CoSO_4.7H_2O$).

5. Addition of a small amount of concentrated sulphuric acid (0.5 c.c. to 100 c.c. of standard) does not change the color of the cobalt standard and helps preserve it.

6. Serum bilirubin reactions do not appear to change on standing if refrigerated.

REFERENCES

[1]Mann, Frank C., and Bollman, Jesse L.: Liver Function Tests, Arch. Path. and Lab. Med. 1926, i, No. 5, 681.

[2]Rosenthal, S. M.: Discussion on Liver Function, Jour. Am. Med. Assn., Nov. 14, 1925, lxxxv, 1549.

[3]Mann, Frank C.: Modified Physiologic Processes Following Total Removal of the Liver, Jour. Am. Med. Assn., Nov. 7, 1925, lxxxv, 1472.

[4]Bockus, Henry Leroy, and Shay, Harry: Obstructive Jaundice, Med. Clin. No. America, September, 1926, p. 379.

[5]Smyth, F. S., and Whipple, G. H.: Bile Salt Metabolism, I, Influence of Chloroform and Phosphorus on Bile Fistula Dogs: Jour. Biol. Chem., April, 1924, lix, 623. Whipple, G. H.: Origin and Significance of Constituents of Bile, Physiological Review, July 1922, ii, 440.

[6]McNee, J. W., and Keefer, Chester S.: The Clinical Value of the van den Bergh Reaction for Bilirubin in the Blood, Brit. Med. Jour., July 11, 1925, No. 3367, p. 52.

[7]Ravdin, Elizabeth Glenn: An Estimation of the Clinical Value of the van den Bergh Test, Am. Jour. Med. Sci., clxix, p. 850.

[8]Andrews: Lancet, London, 1924, i, 590 (as quoted by McNee)[6].

[9]Hall, W. W.: Useful Hepatic Function Tests, Naval Medical Bulletin, October, 1926, xxiv, 4, p. 843.

NOTES ON SICKLE CELL ANEMIA*

By George S. Graham, M.D., and Sarah H. McCarty, A.B., Birmingham, Ala.

IN 1910 Herrick described a case of severe anemia in which the red cells were elongated and sickle shaped.[1] During the following thirteen years, three similar cases were recorded by Washburn,[2] Cook and Meyer,[3] and Mason.[4] In all of these cases the arresting sign had been a peculiar deformity of the red cells as observed in the blood film. The clinical findings associated with the erythrocytic anomaly seemed to outline so definite a syndrome that Mason wrote of his case as an example of "Sickle Cell Anemia."† While studying the case of Cook and Meyer, Emmel[7] found that the cell deformity was greatly accentuated when a fresh blood drop was sealed beneath a cover slip and examined after a lapse of some hours. Not only did the deformity increase but the percentage of affected cells mounted rapidly. In fresh preparations from the case studied about one-third of the cells were elongated, curved or crescentic. In sealed preparations the deformity was greatly exaggerated and the number of affected cells increased until in twenty-four hours practically 100 per cent of the red cells had become converted into various bizarre shapes. (Fig. 1.)

The first real understanding of this curious condition dates from 1923 when two illuminating papers were published, one by Sydenstricker, Mulherin and Houseal,[8] the other by Huck.[9] Following out certain leads from earlier work and adding further findings of their own, they demonstrated that an anemic syndrome characterized by sickling deformity of the red cells is relatively common and is not, as had been supposed, a medical oddity. They verified its striking familial character and both were able to collect cases readily by examining family groups introduced into attention by the discovery of an affected member. These papers focused attention sharply upon the condition and it is now widely recognized, at least among physicians of the South. But its real frequency is not yet fully appreciated.

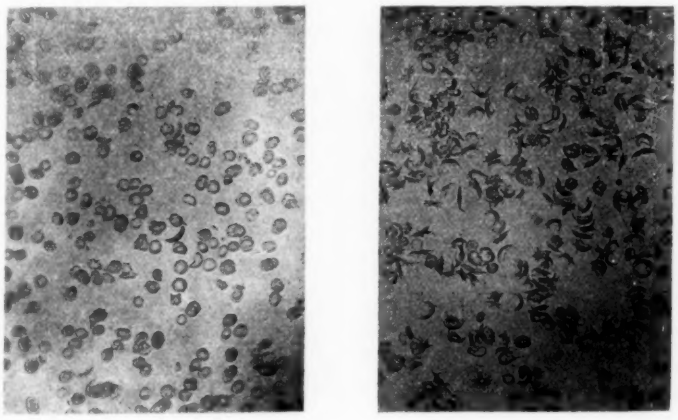
Thus far the red cell anomaly has been demonstrated only in the full or part-blood negro. Sydenstricker[10] is quoted as having examined 1000 white patients without finding a single instance. We have made no systematic attempt to follow this line of inquiry. We have set up occasional preparations from cases of various blood diseases, however, with negative

*Read at the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April, 1926.

†In 1904 Dresbach[5] reported the occurrence of elliptical red cells in the blood of a mulatto medical student. No other abnormalities were found in the blood and there was no clinical evidence of anemia. Dr. Dresbach has kindly furnished us a photo of the stained blood film from his case. He states that cover slip preparations were made of the blood for purposes of class demonstration and that some of these were examined at intervals probably as long as fifteen hours after mounting, but that no further evidences of form change were seen. Bishop recorded a similar erythrocytic deformity in 1914[6]. The patient's race was not stated. The subsequent history of Dresbach's patient is suggestive of that common in sickle cell anemia but it is doubtful whether either of these cases was an instance of the disease.

results. Should continued study fail to show the occurrence of the anomaly in the white race there still remain interesting questions as to its possible occurrence in other races and as to its rate of incidence and its manifestations in the black populations of other parts of the world. Information along these lines would be of considerable interest.

The clinical aspect has been so well covered by Sydenstricker and by Huck that there is no occasion for detailed statement here. Common signs and symptoms are: anemia with its concomitant malaise and disability; general bodily underdevelopment, perhaps accompanied by a subnormal mentality; epigastric pain and low grade gastrointestinal disturbance; muscular or arthritic pain of variable location; greenish coloration of the sclera. General lymphadenoid enlargement occurs sometimes and there may be slight enlargement of the liver. The urine has a low fixed specific gravity. Albuminuria is usually found and there may be cylindruria. Urobilin is usually present. Bilirubinemia is very common in well developed cases and there



A. *B.*

Fig. 1-*A*.—Blood from a case of sickle cell anemia as observed immediately after it had been sealed beneath a cover slip.

B.—A similar preparation as it appeared twenty-four hours later. Red cell deformity or meniscocytosis is now well developed.

may be jaundice. Chronic ulcer of the legs or ankles may occur, particularly after childhood.

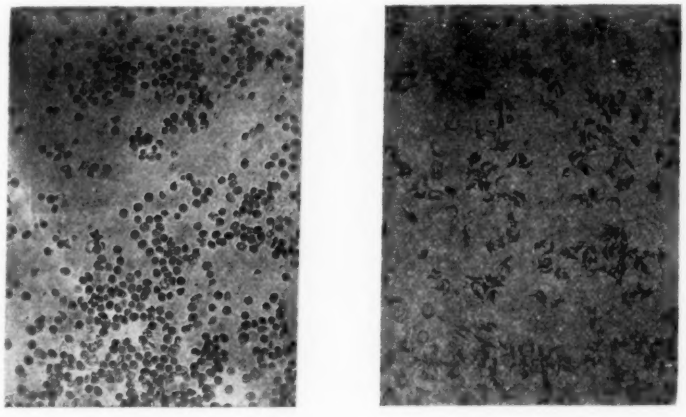
The sickling anomaly may be found in individuals who declare themselves well and strong and who give no history of any illness attributable to the blood dyscrasia. Sydenstricker regards such instances as examples of a "latent phase" of the disease. In the majority of cases the affected individual presents some, at least, of the stigmata above outlined while in occasional individuals the condition becomes of major importance. There is now well marked anemia. The patient is "puny," listless and easily fatigued. At intervals of weeks, months, or years, there are attacks of acute illness with exacerbation of the anemia and its attendant conditions. There are many evidences of active blood destruction. It is of particular interest that such attacks in the susceptible person may follow exposure to cold or damp-

ness. They may perhaps be brought on by overexertion. In our experience these attacks are associated with evidence of some infection, particularly of the respiratory tract. Huck was impressed with the susceptibility to pneumonia and tonsillitis, an opinion with which we agree. In symptomless cases the sickling deformity may be the only definite abnormality of the blood. There is, however, apt to be some anisocytosis and polychromasia. Such abnormalities may become marked and may be associated with poikilocytosis and the appearance of erythroblasts even in individuals who make little complaint of ill health. The white count is usually elevated, markedly so in active cases and here a few myelocytes appear. Moderate eosinophilia is common. There is a high percentage of reticulocytes during exacerbations, together with large numbers of nucleated red cells. In an active case we once encountered 762 nucleated red cells in differentiating 500 leucocytes, while reticulocytes ran as high as 30 per cent. Phagocytosis of the red cells by circulating endothelial leucocytes is a common phenomenon. It may occur even in the absence of complaint and in patients who have good red cell counts and hemoglobin values.

Even as brief an account as the above may serve to indicate how serious a problem a condition of this sort may become, particularly when it is remembered that the affection is not at all uncommon. These individuals bear the constant burden of a blood dyscrasia whose outstanding feature is an abnormally high destruction rate of the erythrocytes. With the advent of periods of stress such as may accompany infection or other untoward circumstance, they are correspondingly handicapped. In medical, surgical, and obstetrical wards these are the patients who stay longest in the hospital and are most liable to complications and sequelae. They appear to be relatively short-lived. Among fifty-eight cases that have come under our observation, only two have been over fifty years of age. One of these was fifty-six, the other fifty-seven, and both were of the inactive type. Five of the series were newborn infants; four were children in the first decade of life; twelve were from eleven to twenty years of age, twenty-nine from twenty-one to thirty years of age, three from thirty-one to forty, three from forty-one to fifty. The marked preponderance of patients in the second and third decades may be explained in part, though not completely, by the greater relative number of such individuals making up the general hospital population. We do not yet know whether the condition may be recovered from in later life. So far as present knowledge goes, this seems very improbable and we are forced to assume with Huck that most of these people die before they have passed the thirtieth year.

We have been surprised at the frequency with which the sickling deformity can be demonstrated. At different periods, each of several weeks' duration, we have set up routine cover slip preparations on all negro patients on whom blood counts were being made. Medical, surgical and obstetrical patients were included. We have thus examined the blood of 608 individuals. Among them we have encountered forty-four instances of the sickling deformity. For this series, then, the anomaly is demonstrable in 7.2 per cent of the patients in the negro wards of a county hospital. In an earlier summary drawn

from 250 patients we found 5.2 per cent of "sicklers." We have attempted to follow family groups in only a few instances but among eleven immediate relatives of known sicklers eight were positive. Of six infants born of sickling mothers, five have been positive. Sydenstricker[10] reports an incidence of ¼ of 1 per cent among the negroes examined at his clinic. His series is much larger than our own and it included a large number of out-patients, that is, of individuals probably suffering from minor complaints, but the disparity in the incidence rate is still striking. It is possible that our higher rate is due to our routine use of thin cover slips in the setting up of preparations. Certain puzzling experiences of our earlier work lead us to look for a purely physical explanation of the cell deformity and after various attempts at altering the physical and chemical conditions to which the cells were exposed in vitro, we found, among other things, that a difference in the thickness of the cover slip under which they were compressed might promote



A. *B.*

Fig. 2.—Two cover slip preparations were made simultaneously from the same patient and upon the same slide. "*A*" was covered with a No. 2 cover slip; "*B*" with a No. 0 cover slip. Photographed twenty-four hours after set-up.

or hinder deformity. A thin cover may elicit sickling while a thick one fails to do so. We have repeatedly found that when similar drops of blood are placed at opposite ends of a slide and covered, one with a No. 0 ⅞ inch cover square, the other with a No. 2 square of the same size, sickling appears promptly under the thin cover but is absent under the thicker (Fig. 2). The difference is not so marked when No. 0 and No. 1 covers are used but it is still present in an occasional blood. In searching for the anomaly we have always used the No. 0 cover slip and have thus recorded occasional positive findings where thicker covers would have given negatives. In many of our cases, as a matter of fact, both thick and thin covers have been used.

In one case where sickling was active, peculiar form changes were observed by watching the cells during the critical period of break-up. While the red cell of normal blood appears homogeneous and firm, it seemed here soft and unstable. Its substance appeared agitated by a play of forces that kept the surface membrane and contents in a continued state of instability.

Areas of condensation or folds developed slowly or suddenly at the periphery and zones of rarefaction appeared alternately with fields of condensation in the cytoplasm so that the cell contents appeared to separate into globules or segments. Indentations or deep clefts developing at the surface were refilled by a sudden inrush of cytoplasm. The patterns produced by this slow kneading of membrane and contents were kaleidoscopic. A favorite formation was the development of equatorial or paracentric fissures one or more in number, often set at right angles to each other or arranged as multiple radiations from the middle region so as to set off several unequal sectors. The constantly changing contour was apt finally to develop a unilateral condensation or crescent and finally the tension set up somewhere at the surface appeared to become too great to be sustained and with a sudden snap the cell opened out into the characteristic "sickle" or changed more gradually into some irregular burred form while the cytoplasm flowed out at the ends or from multiple points about the periphery into thorny prominences or long, delicate streamers. The cell membrane or surface layer might appear to be retained, although it became wrinkled or stretched out into unusual shapes like an old piece of rubber that had lost its elasticity. But in some cases at least, the collapsed shell seemed to be partially or completely thrown off and the inner substance spread itself out irregularly.

We have not observed sickling in red cells that had been received into large volumes of various isotonic salt solutions, buffered or unbuffered, and set up in high dilution in such fluids. After centrifugation, however, sickling has been seen when the packed cells have been set up in these fluids without dilution. Here it is noticeable that the deformity begins about the periphery of platelet and leucocyte clumps, spreading slowly from such centers to involve wider fields. Washed cells sickle slowly and uncertainly in serum, but more normally in plasma. Defibrinated or oxalated blood also sickles slowly or imperfectly and again the change begins about platelet-leucocyte clumps. We have reached the tentative conclusion that the red cell deformity is determined in vitro by optimum pressure conditions plus biochemical activity of serum or plasma constituents. There is a strong suggestion that it is in some way related to the development of the fibrin net.

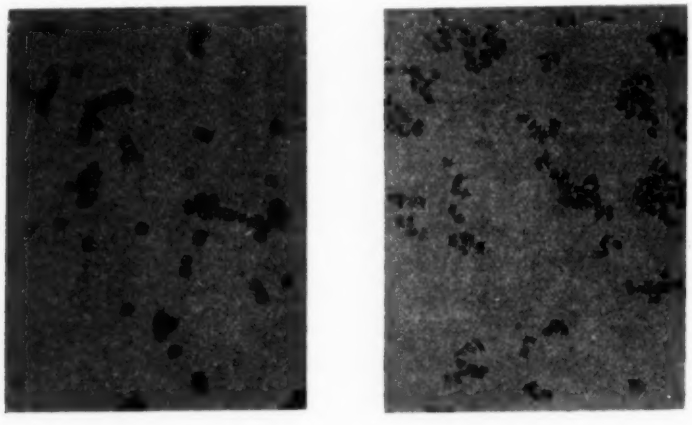
Evidence of a peculiar red cell structure is furnished by the findings of the "fragility" test. In several respects there are resemblances between sickle cell anemia and hemolytic jaundice. Here, however, there is sharp contrast. The red cells are found to have an increased rather than a diminished resistance to the hemolytic action of hypotonic salt solutions. Determinations made on twenty-four different individuals, some symptomless, others presenting various degrees of anemia and disability, have shown consistently low values for the point of complete hemolysis. This increased resistance is, in our experience, one of the most constant phenomena encountered, whether in active or in latent cases. Hemolysis begins at about the normal level. The highest value found was 0.48 per cent, the lowest, 0.325 per cent, with an average for all cases of 0.395 per cent. The highest level at which hemolysis was complete was 0.28 per cent, the lowest, 0.12 per cent, the average for the whole series being 0.19 per cent. In three cases the point of complete

hemolysis appeared to run lower than the levels quoted but in the absence of confirmatory readings these have not been included in the above figures.

The sedimentation rate of the red cells is undoubtedly increased. We have made a few rough determinations on oxalated blood that had been collected as for routine blood chemistry. We used the simple expedient of drawing 1 c.c. of this blood into a 1 c.c. serologic pipette and measuring the amount of fall in the red cell column at ten minute intervals. The distance between the original level and the top of the descending red cell column was converted into percentage of the original total height. When thus set up alongside normal controls, the settling rate of affected bloods is strikingly increased. Actual figures for one set-up showed for the normal control a percentage decline of the red cell column during successive ten minute intervals of the first hour of: 0.4; 1.3; 3.7; 6.9; 9.3 and 10.2. The blood of two obstetric patients taken during the puerperium showed: 1.0; 5.3; 15.0; 22.3; 32.5; 37.5 in one case and 6.3; 11.3; 21.9; 31.3; 34.5; 38.7 in the other. That of a third (medical) patient showed 9.0; 22.5; 50.0; 55.0; 57.5; 60.0. This blood showed no further fall at eighty minutes and at two hours. The normal stood at 13.4 at eighty minutes and both obstetric patients at 42.5. At two hours the normal stood at 17.6 and the obstetric patients at 47.7 and 48.0.

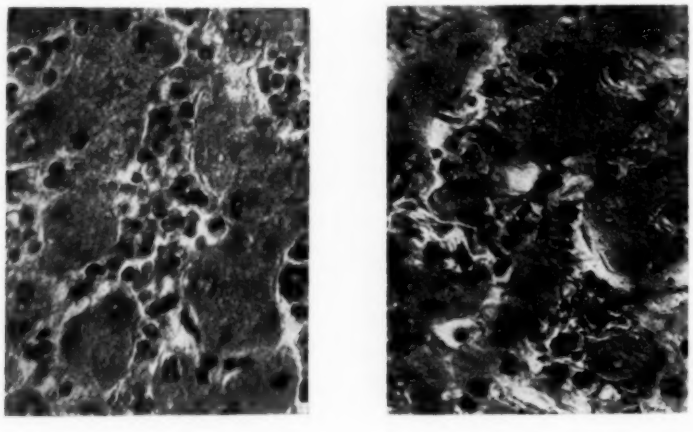
Chemical determinations have been run on both active and inactive cases. The nitrogen values were disregarded after normal figures had been found in the earlier cases. Study of the inorganic constituents was continued for a longer time and determinations have been made in whole or in part on twenty-two patients. Chlorides have been determined in twenty patients. The amount in the whole blood (calculated as NaCl) ranged from 310 to 594 mg. per 100 c.c. of blood, the average for the whole series being 501 mg. Calcium varied in fourteen cases from 6.0 to 11.8 mg., with an average of 8.33 mg. Inorganic phosphorus in eighteen cases of varying age ranged from 2.04 to 6.45 mg. We have been unable to establish any relationship between the degree of anemia or the severity of complaint and the level of these blood constituents. There is, on the other hand, a marked disturbance of the cholesterol. Thirteen determinations have been made by the method of Bloor. The lowest value found was 208 mg. per 100 c.c. of blood. Two girls each seven years of age gave identical readings of 222 mg. Both were known sicklers who had been in the hospital, one ten months, the other one year, previously, and were visited at their homes for purposes of check. Both were playing about normally and both disclaimed any complaint, although one showed a red count of 3,560,000 and a hemoglobin of 48 per cent (Sahli), the other a red count of 3,440,000 with 45 per cent hemoglobin. Four patients showed cholesterol of 260 to 273 mg., three a value of 300 to 333 mg., one had 450 mg., one 490 mg., and one obstetrical patient who had been delivered eight days previously had a value of 877 mg. This girl was fifteen years of age, had a red count of 3,390,000, hemoglobin 60 per cent, W.B.C. 6900. Three nucleated red cells were seen in differentiating 100 leucocytes. Sedimentation rate was not taken. Included in the series just quoted were four other obstetric patients, all examined at intervals of four to thirteen days after delivery and showing cholesterol readings of 266, 333, 333 and 490 mg.

We have had opportunity for making postmortem examination in four cases. A study of the first of these has already been reported.[11] None of the subsequent cases have been so well developed but each has been valuable as affording means for comparative study. A detailed histologic study of these cases will be reported upon at a future date but we wish to call attention at this time to certain findings that appear to us to have a possible bearing upon the general understanding of the condition.



A. *B.*

Fig. 3.—Cover slip preparation of blood taken from a vein during autopsy. "*A*" was photographed one hour after set-up; "*B*" about twenty-four hours later.



A. *B.*

Fig. 4.—Sections from liver (Case II). "*A*" is from a block fixed in Zenker's solution; "*B*" from a block fixed in formalin. Both blocks were imbedded in paraffin, the former by the chloroform method, the latter by the simpler benzol method.

Of the four cases in the series, the first two had a similar clinical course. In Case I, a male, aged thirty years, death was due to streptococcic bronchopneumonia with associated bacteremia. Case II was that of a male, aged nineteen years. Death was again due to acute bronchopneumonia. In this case, as in the first, the lungs showed many small abscesses developing in

areas of peripheral infarction. Streptococci were present in these lesions, as well as in the pleural cavity. The autopsy was performed twenty-seven hours after death. Blood taken from a vein during autopsy and sealed beneath a cover slip in the usual manner showed at first only occasional deformed red cells but next day there was typical sickling deformity in about 75 per cent of the cells. (Fig. 3.) The same observation had been made in the first case. As in the first case also, it was found that paraffin sections of tissue fixed in Zenker's solution contained red cells of natural appearance, whereas parallel blocks from formalin-fixed tissue showed well marked sickling deformity or at least an equivalent distortion of the cells. (Fig. 4.) Case III was that of a truck driver, aged twenty-nine years who died of meningeal hemorrhage three days after the receipt of accidental cranial injury. The sickling deformity of his red cells was discovered only as the result of the routine examination that was being practiced on unselected patients. He had a red count of 4,360,000 and a hemoglobin of 90 per cent, the highest values for any patient in our series. In Case IV the age was twenty-three years. The patient had a positive Wassermann and cutaneous scars of a secondary luetic eruption. He died of peritoneal and generalized tuberculosis after an illness of about three months' duration.

The one finding common to all these cases was, as might be expected, the evidence of a continued erythrocytic destruction-rate higher than the normal. Such evidence consisted, first, in the widespread deposit of hemosiderin in the tissues, most marked in the spleen but present also in liver, bone marrow and lymph nodes. There was also definite hyperplasia of the bone marrow in the first two cases. The marrow was not obtained in Case III. In Case IV, the marrow of the femur showed well-marked serous atrophy. In all four cases many phagocytic cells containing red cells or blood pigment were found in the great reticuloendothelial centers and such cells occurred also in occasional vessel lumina of various organs.

DISCUSSION

Following present usage, we have labeled as "sickle cell anemia" every case in which sickling deformity has appeared in the red cells. But as knowledge of the condition increases it will probably become advisable to restrict the use of this term. In some of our cases there has been no clinically recognizable evidence of anemia at the time of examination. We believe that in the present state of our knowledge it would make for clarity were we to look upon this cell deformity merely as one demonstrable stigma of an inheritable blood dyscrasia or status upon the basis of which there may, under conditions not yet understood, be built an anemic syndrome of well characterized type. If the problem be thus conceived, there is need for some word that may serve to designate the presence in a given blood of the sickling anomaly without at the same time committing us to any decision on the further question as to what other morbid changes may have made their appearance in the given case. To fill such need we have devised and would offer for adoption the term "meniscocytosis." It is derived from the Greek "meniskos," meaning "a sickle." As used, it would designate a peculiar and apparently

unique type of poikilocytosis dependent on familial and probably on racial abnormalities of the red cells or of their suspending fluids or of both; partly inherent in the fresh blood cells but fully developed only under artificial conditions. We believe that the occurrence of this meniscocytosis in the blood of a given individual need not necessarily imply the presence of any clinically demonstrable anemia but it should at least serve as a warning signal. The affected individual is at once included in that 5 to 7 per cent fraction of the population in the negro wards which deserves special care in treatment and prognosis. As stated above, these individuals appear very susceptible to infectious disease and particularly to infections of the respiratory tract. Among the patients of the present series there has been a high incidence of tonsillitis, bronchitis and pneumonia. Of four deaths in affected persons, two were due to bronchopneumonia. The condition is of serious import in children, as was pointed out by Sydenstricker in his first paper. In the present series of cases from the wards of the Hillman Hospital, a charitable institution accepting everything except contagious diseases, 50 per cent of the patients have fallen in the age group of twenty-one to thirty years. The condition would appear also to be of serious consequence to the young adult.

We have as yet no explanation for the meniscocytosis. The freshly drawn blood may show abnormalities in the size, shape and staining qualities of the red cells. These may become so marked as to be diagnostic, particularly when, as is not uncommon, they are accompanied by erythroblasts. But on the other hand they are often so slight as easily to escape notice. Yet in the latter case, as in the former, sealed preparations may develop extreme deformity. The phenomenon is probably dependent on a peculiar lability of the erythrocytic cytoplasm. It may be influenced readily by variations in the physical conditions to which the cells are subjected in vitro, as is evident from the above note on the different reactions obtained through variation in the cover glass thickness. Such differences probably depend upon the securing of optimum pressure conditions. It is probable that another factor in the production of distortion is the lateral traction exerted upon the individual cells by adherent fibrin filaments formed during coagulation. Neither force is potent when acting alone. Together they produce distortion. Red cells acted upon within the tissues by fixing fluids may preserve their rounded shape in the presence of a "good" cytologic fixative such as Zenker's solution but may suffer distortion under the unequal strains produced by the shrinking action of a "poor" fixative such as formalin.

Biochemical factors may contribute to the erythrocytic abnormality that makes deformity possible. The red cells of these individuals exhibit a striking rouleau formation and there is often a distinct granularity in cover slip preparations with the appearance of clumps in which the rouleaux are irregularly formed or heaped together. When the cells are separated from their plasma or serum and subsequently remixed, this granularity becomes more marked, particularly when the materials have been chilled and the preparations are kept at low temperature. Sydenstricker[12] states that he has observed undoubted autoagglutination in four cases. No very clear-cut results have been

obtained by previous workers through crossing red cells and serum or plasma of normal and of affected individuals nor have the few trials made by us along this line been entirely decisive but it is probable that in the "sickler's" blood there is abnormality both of the cells and of the plasma bodies. In our first case, we were impressed by the patient's repeated statement that he was particularly liable to periods of illness after he had been exposed to cold or dampness and that his brother suffered similar disturbance under like conditions. His fatal seizure began the day after a snowstorm during which he had become cold and wet. There was here a distinct suggestion that the attacks were of a nature similar to those of paroxysmal hemoglobinuria. But the latter disease may occur in the negro without the appearance of meniscocytosis, as we had opportunity to observe while the study of this first case was in progress. The failure to find the red cell anomaly in a condition marked by such profound disturbance of the serum-immune bodies as is present in that disease lead us to doubt the influence of such bodies on the sickling deformity and to prefer to it the hypothesis that the influence of exposure might more simply be explained as predisposing to infection and that infection merely emphasized a latent blood anomaly. But as we have had from an occasional patient the repetition of this belief in the predisposing action of exposure and particularly as we have been more impressed by the observation of the pseudoclumping if not actual autoagglutination in repeated specimens of fresh blood, we have been inclined to return to the earlier idea that serum anomaly may be present and significant. It must still be remembered that autoagglutination will not explain all the phenomena of the condition. We have recently had under observation a white woman whose blood contained autoagglutinins. She suffered a long and difficult convalescence from puerperal septicemia and exhibited a profound anemia but her red cells never showed any evidence of meniscocytosis.

In our present series of four autopsied cases the histologic finding that seems to be most constant and most significant is the widespread occurrence of a rather active phagocytic destruction of the erythrocytes. It is prominent even in Case III, that of a truck driver apparently healthy and possessing a normal red count and hemoglobin, who died three days after the receipt of accidental cerebral injury. It will be recalled that endothelial leucocytes containing red cells are common in the circulating blood during life. They were first noted by Emmel. Sydenstricker states that they may be found in every case if carefully looked for. Judging from the number of these cells found in sections of the liver, spleen, bone marrow, and lymph nodes, as well as in occasional vessel lumina of various organs, the actual number of erythrocytes constantly in process of destruction must be considerable. This finding recalls the condition that has been described for hemolytic jaundice as well as the marrow changes in pernicious anemia discussed recently by Peabody.[13] We were first inclined to look upon this phagocytosis as evidence of infectious injury of the red cells. A streptococcus had been cultured from the heart blood of our first case and great numbers of chained cocci were seen in sections from the pulmonary lesions. The pulmonary changes in Case II were very similar to those of the original case

and like them contained a streptococcus. In Case IV death was due to tuberculosis. But in Case III there was no evidence of infection yet here also there is active phagocytic destruction of the red cells in the great reticuloendothelial centers. Despite the presence of meniscocytosis this patient presented no evidences of "sickle cell anemia." It would appear, then, that phagocytic destruction of the red cells may occur in the absence of any evident infection and that it may also occur without producing any of the usual clinical evidences of anemia. Meniscocytosis and endothelial erythrophagocytosis constitute, perhaps, anatomic expressions of a fundamental blood dyscrasia accompanied by or dependent upon the presence in the plasma of a body inimical to its own red cells. The hypothesis offers a rational basis for the explanation of the unquestioned inheritability of the condition. Given an individual thus constituted, we may explain such phagocytic destruction of the red cells as is found in Case III, we may explain also the hyperplasia of the bone marrow observed in our cases and reported also by Sydenstricker and by Huck. Such hyperplasia may compensate for the constant drain upon the formative cells and so maintain normal values for the circulating blood. But in some cases it is unable to do this. The individual must then accustom himself to a lowered erythrocytic volume. He is handicapped under conditions of special stress and falls ready prey to the attack of toxic or infectious noxa. The surprisingly high hospital admittance rate for individuals in the third decade of life may indicate that at this age period the bone marrow begins to fail under the burden of its years of work hypertrophy. So we find in Case IV the gelatinous marrow of old age or long continued exhaustion making its appearance in a young man. The fatal illness in this case would hardly be expected to produce such extreme exhaustion of the marrow under ordinary conditions.

The increased cholesterol content of the blood is of considerable interest. Several of our high values were obtained in puerperal women, but similar figures were found in other adult patients, and also in children who were free of complaint although definitely anemic. This high level contrasts sharply with the lowered values common in other anemias and particularly with that found in pernicious anemia. It might be suggested that in view of the antihemolytic property of cholesterol the increase in its amount in the blood may represent some reaction of defense against the active erythrophagocytosis. The validity of such a theory would, however, be raised in question from the fact that an argument essentially the opposite has been advanced to explain the increased blood destruction in pernicious anemia.

The general relationship of sickle cell anemia to other diseases of a similar nature is not yet established. Under the designation of "Herrick's Anemia" Ward has suggested the inclusion of the disease as one type form in his "Anemias of the Hemolytic Jaundice Group."[11] His paper develops the conception of a group of diseases dependent fundamentally on a congenital or hereditary plasma defect. In at least some members of the group the abnormality of the plasma consists in the presence therein of an autoagglutinin. Whatever its nature, the plasma defect renders the red cells "more prone to destruction, either by the normal mechanism of portal

hemolysis, or by phagocytosis, or by both.'' In hemolytic jaundice there is an almost purely hemolytic anemia, in ''Malin's syndrome,'' an almost purely phagocytic anemia; while both types of cell destruction are present in Herrick's anemia. Outside hemolytic jaundice, Ward has been able to collect but few cases illustrative of his type forms but his conception is interesting. Certainly, in the light of our present knowledge, Herrick's or sickle cell anemia seems, despite its divergences, more closely allied to hemolytic jaundice than to any other commonly recognized syndrome.

REFERENCES

[1]Herrick, J. B.: Arch. Int. Med., 1910, vi, 517.
[2]Washburn, R. E.: Virginia Med., 1911, xv, 490.
[3]Cook, J. E., and Meyer, J.: Arch. Int. Med., 1915, xvi, 644.
[4]Mason, V. R.: Jour. Am. Med. Assn., 1922, lxxix, 1318.
[5]Dresbach, M.: Science, 1904, xix, 469, ibid, 1905, xxi, 473.
[6]Bishop, F. W.: Arch. Int. Med., 1914, xiv, 388.
[7]Emmel, V. E.: Arch. Int. Med., 1917, xx, 586.
[8]Sydenstricker, V. P., Mulherin, W. A., and Houseal, R. W.: Am. Jour. Dis. Child., 1923, xxvi, 132.
[9]Huck, J. G.: Bull. Johns Hopkins Hosp., 1923, xxxiv, 335.
[10]Sydenstricker, V. P.: South. Med. Jour., 1924, xvii, 177.
[11]Graham, G. S.: Arch. Int. Med., 1924, xxxiv, 778.
[12]Sydenstricker, V. P.: Jour. Am. Med. Assn., 1924, lxxxiii, 12.
[13]Peabody, F. W., and Broun, G. O.: Am. Jour. Path., 1925, i, 169.
[14]Ward, Gordon: Proc. Roy. Soc. Med., 1919-20, xiii, Pt. 1 and 2, Sec. Med., pp. 1-21.

A COMBINED DILUTING AND STAINING FLUID FOR DIFFERENTIAL LEUCOCYTE COUNTS IN THE COUNTING CHAMBER*

By Daniel Nicholson,† Winnipeg, Canada

THE most widely used method for differential leucocyte counts is the spreading of a drop of blood on a glass slide which is examined under the microscope. A great concentration of polymorphonuclear cells will be found at the margin and at the end of the smear. Many authorities recommend examining in a zigzag manner across the slide so as to take in the polymorphs at the edge and lymphocytes in the center of the smear. No suggestion is made regarding how far one should go into the body of the smear where most lymphocytes are met. A worker who examines half an inch into the body of the smear will obtain a higher lymphocyte percentage than one who examines in only a quarter of an inch. Some recommend examining right across the slide. By any of these methods the accumulation of polymorphonuclear cells at the end is left out entirely so that an accurate differential count is impossible. In the cover glass spreads the distribution of the leucocytes is more uniform but for some reason, perhaps because of technical difficulties, this method, although it originated many years ago, has never been generally adopted.

The cells in the counting chamber are evenly distributed and if a satisfactory stain is mixed with the diluting fluid one can distinguish the different forms of leucocytes and so make a differential count.

Dunger used ½ per cent eosin in 10 per cent acetone to stain the eosinophiles in the counting chamber. Later Dr. Stitt in the sixth edition of his excellent textbook on practical bacteriology used Giemsa's stain added to a weak neutral formalin solution, as a combined diluting and staining fluid for leucocytes. With this, the leucocytes were well stained but had a fuzzy outline and the red cells were only partially laked. The procedure is omitted in the seventh edition of this textbook.

Frequently a clinician requires a total and differential leucocyte count from the one patient without any information regarding the red cells. By the counting chamber method we could make a total count of their number per cubic millimeter and by examining fifty microscopic fields under the high power lens, an accurate estimate of the various types of cells could be made. Thus the total and differential leucocyte count could be done in less than half the time required by our present methods.

There are many difficulties in obtaining an ideal combined diluting and staining fluid. There must be no clotting or clumping of the blood. The red cells have to be hemolyzed or rendered transparent. The fluid must

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16, and 17, 1926.

†Assistant Professor of Pathology, University of Manitoba, Winnipeg, Canada.

hold the stain in solution in sufficient concentration that it will stain the cells in a few minutes in such a manner that the various types may be recognized. It must have a specific gravity less than the cells or they will float about instead of resting on the bottom of the counting chamber. Each one of these requirements can be readily obtained singly but I have spent many hours indeed trying to find a fluid that would combine all these features.

The most convenient and material saving technic I have found is: Place a large drop of the diluting fluid on a glass slide under the low power of the microscope. Add a small drop of blood by means of a platinum loop and notice if there is any clumping of the cells, coagulation of the protein or hemolytic bleaching action on the red cells. If no clumping of the cells or clotting of the protein takes place and the red cells become invisible but

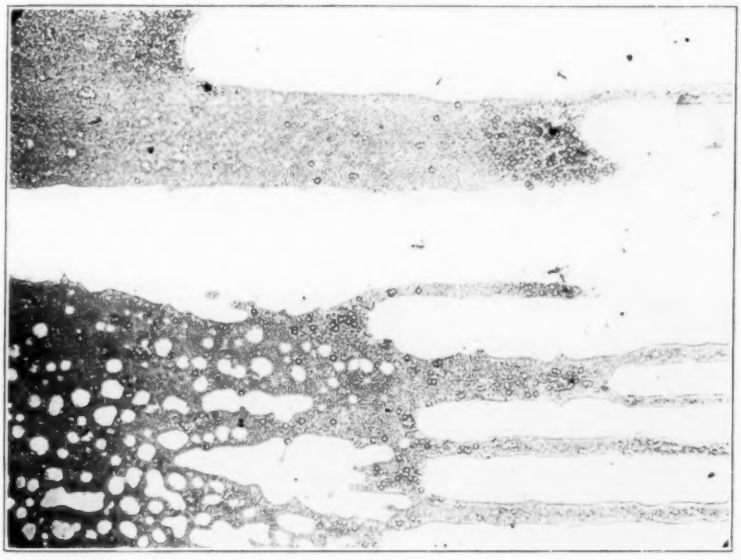


Fig. 1.—Photomicrograph of the end of a blood smear. Note the large collections of polymorphs which appear as small dark circles and the scarcity of these cells farther in.

the leucocytes remain uninjured, place two more large drops on a glass slide. To one add a small amount of nuclear stain by means of a toothpick and note the intensity of the solution seen under the microscope as a test of solubility. Treat the second drop with a cytoplasmic granular stain such as eosin or acid fuchsin and observe in the same manner. Mix a part of each drop and if a precipitate occurs, it can be readily seen under the microscope. To all three drops add a loopful of blood and observe the degree of staining under the microscope. I tried all the possible combinations I could think of in this manner. This technic is very simple, rapid and satisfactory. If clumping did not take place and some staining of the cells occurred, I made $\frac{1}{10}$, $\frac{1}{4}$, $\frac{1}{2}$ and 1 per cent dilution of the powdered stain in the laking fluid and used it to dilute the blood in the leucocyte pipette. Here I might mention that if one is not using a dilution pipette of this type constantly, it is difficult to draw the blood up to the correct mark and no farther. If it

is drawn up farther and blown back, a coating remains on the tube which makes for inaccuracy. I have spent considerable thought on a pipette to overcome this and of course a syringe arrangement suggested itself very soon. I found out later that Pappenheim had brought one out on this principle in 1910. It was not satisfactory because the bore of the pipette is so small compared with that of the syringe that, with the slightest syringe movement, the blood moves quite rapidly and is liable to pass the mark. To overcome this, I devised a bevelled lower end of the syringe so that by turning it, a very slight backward movement, just enough to draw up the column of blood slowly, is obtained. For the diluting fluid the syringe may be drawn back in the ordinary way. The error of not being able to stop exactly at the upper mark causes only one-twentieth the error of a miss at the lower mark.

With the diluted blood in the pipette, after one minute's shaking I

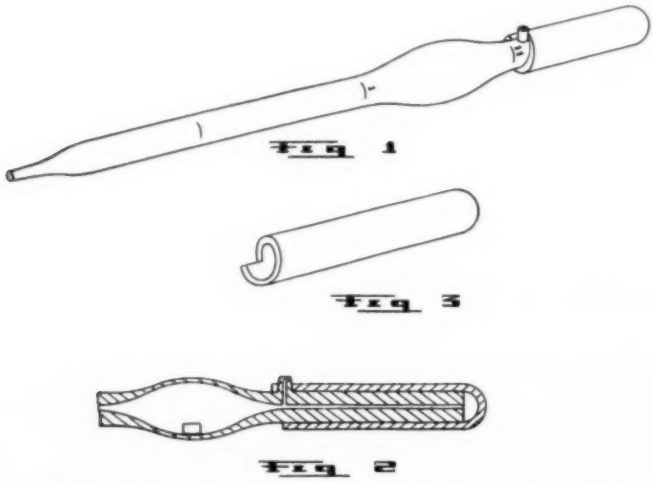


Fig. 2.—Precision leucocyte pipette, *Fig. 1*. Assembled, *Fig. 3*. The cap with beveled proximal end which when turned produces a slow and steady backward motion. *Fig. 2*., a cross section of the pipette. To obtain a vacuum the thumb must be placed on the knob while the cap is drawn back.

placed a drop in the counting chamber and examined it under the microscope at intervals of three, five, ten, and fifteen minutes from the time of filling the pipette, recording the results in each case.

The best hemolytic substances I found were saponin and sapotoxin but the stains dissolved in them did not produce a clear-cut outline of the cells. All the benzine substances were tried but as they were very poor stain solvents they had to be given up. Watery solutions did not hold many of the stains well and any substance which merely produces a rupture of the envelope of the red cells was unsatisfactory because the ruptured fragments partly stained, were strewn about the microscopic field and obscured the view of the leucocytes.

The most satisfactory type of fluid is one which dissolves out or renders the pigment in the red cells transparent. Acetone will do this and will mix with water. A 10 per cent watery solution will make red cells transparent

but over 25 per cent will coagulate the blood. It will not hold the eosin-methylene blue stains, like Wright's and Jenner's stain solution, in sufficient concentration to dye the cells but will hold Giemsa's stain. The prepared stain contains alcohol and glycerin which retard the action of the acetone on the red cells so that it is necessary to make an increase of the acetone up to 20 per cent to obtain the desired bleaching on the red cells. When these solutions are mixed a slight precipitate occurs, which does not interfere with the examination but may be removed by decanting the supernatent fluid one hour after the stain is added to the acetone solution.

Of all the combinations of diluting and staining fluids I have tried, 6 per cent Giemsa's stain in 20 per cent acetone is the most satisfactory. Keep the

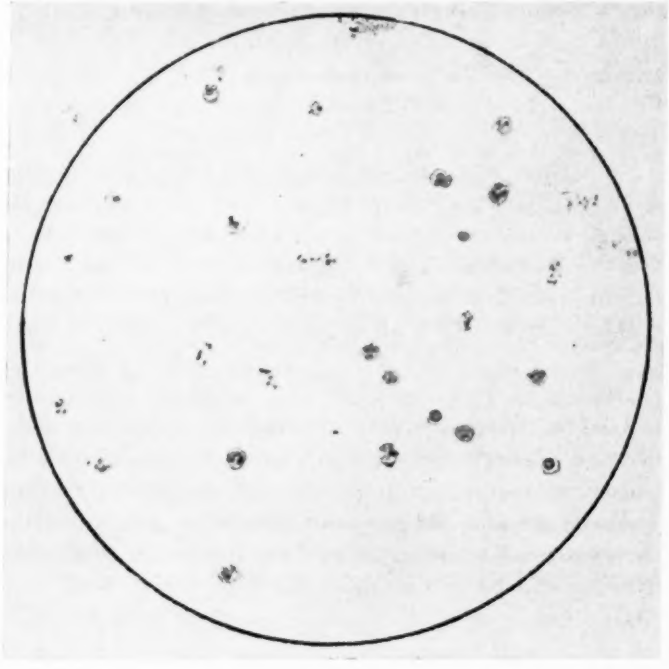


Fig. 3.—Appearance of white cells in the counting chamber under the high power lens when 6 per cent Giemsa's stain in 20 per cent acetone is used as a diluting fluid.

pure Giemsa's stain in one bottle and 20 per cent acetone in distilled water in another. Before making a count add 0.6 c.c. of Giemsa's stain to 10 c.c. of the 20 per cent acetone.

As a distilling fluid, it renders the red cells transparent in five to ten minutes. This can be determined by watching a change in the bulb of the pipette from opaque to transparent. After shaking the pipette, a drop of the contents is placed in the counting chamber and examined under the high dry lens with a full light and *the condenser slightly down.* That most important part of the cell, the nucleus, stands out very clearly. One can readily differentiate the young, solid U-shaped nucleus from the mature segmented ones. This is important in the Arneth or the modified Schilling counts. The mononuclears show a

very faint cell outline. The eosinophiles can be distinguished by their reddish cytoplasm. Crystals of the stain are seen but they do not obscure the view of the cells. The blood platelets are faintly stained and may be seen in clumps. The fluid will keep one day, after which time it stains more slowly.

It is not without its faults and drawbacks however. At least five minutes are required for the solution of the red cells and the staining of the white cells. The pipettes and counting chamber must be scrupulously clean. If the faintest trace of acid reaches the solution the red cells take on a pinkish stain and the nuclei of the leucocytes are very faint. This is the commonest cause of failure as frequently a trace of acetic acid remains in the pipette. This acid reaction can be recognized by noting a change in color of the diluting fluid from deep purple to a greenish blue in the bulb of the pipette.

If the faintest trace of alkali becomes mixed with the fluid, the granules of the eosinophiles will be greenish or if the alkali is strong they will remain altogether unstained. The acetone evaporates more rapidly than a watery solution would, but a total and differential count can be easily done before the evaporation will impair the result. The solution is not a permanent one. The Giemsa's stain must be added to the 20 per cent acetone solution daily.

It would be an important advance to obtain a combined diluting and staining fluid that would not require such exacting precautions. While we are waiting for this, the difficulties mentioned can with a little care be overcome and one can make a reliable differential leucocyte, or modified Arneth count almost as quickly as doing an estimation of the total leucocytes.

SUMMARY

1. The leucocytes are not evenly distributed on a glass slide and it is impossible to do an accurate differential count of them by this method.

2. The leucocytes are evenly distributed in the counting chamber and if 6 per cent Giemsa's stain in 20 per cent acetone is used as a diluting fluid the red cells are rendered transparent and the leucocytes well stained so that a differential count can be made under the high power lens.

3. Even faint traces of acid or alkali interfere with the staining.

4. A fresh mixture of Giemsa's stain and acetone solution must be made daily.

DISCUSSION

Dr. A. H. Sanford.—Dr. Nicholson asked me to open the discussion, but after reading and hearing his paper I feel there is very little to say, as he has covered the ground so thoroughly. He has pointed out the needs of the methods, and also the difficulties encountered.

There is one matter that might be mentioned relative to pipettes. For a number of years I have been interested in improvements of hemocytometer pipettes. The pipette made for A. H. Thomas Co. according to the design of Mr. Trenner is attracting attention. Recently I have also received from England an automatic pipette, the Piney pipette, made by Hawskley & Sons, London, England, which is apparently very accurate.

Dr. Nicholson's ingenious method is worth a trial by all of us.

Dr. B. F. Stout.—I have for many years felt little confidence in differential counts made on slide-smears. The Germans have always insisted that cover-slip spreads be used, and some writers in this country prefer their use. I have for years where a critical differential count was necessary, made the count of the percentage of polymorphonuclears in the

counting-chamber. Here the best distribution of cells may be found. The nuclei are readily distinguished by the high dry power with the dilute acetic acid and careful counts with this method have shown the inaccuracy of the slide-smears. I am very much pleased to have heard Dr. Nicholson's paper and feel sure it is an important advance in the interests of accuracy as well as a time saver.

Dr. F. W. Hartman.—This method is very interesting. I think this Society could well emphasize, as a Society, some of the points Dr. Nicholson has made in regard to these counts.

Why is it that 90 per cent, perhaps 95 per cent, of our students count by this slide method? Why are they not shown the cover-slip method? When they do know the cover-slip method they do not use it.

How does this work on spinal fluid? It sounds as though it might work. I would like to know how long the cells will keep in the pipette. Could we draw the blood this morning and several hours later do the count and get satisfactory results?

Dr. Otto Lowy.—It seems to me that in the discussion some stress was laid upon whether it would be easier to do this than the slide method. I think that in our organization we should place accuracy above rapidity. That is about all I have to say. I wish to complement Dr. Nicholson on his work.

Dr. Philip Hillkowitz.—Sir Almroth Wright in his book *The Technique of the Teat and Capillary Glass Tube* emphasized the inaccuracy of the usual slide method because the polys would be unequally spread if the spreader were not perfectly plane. He has developed a technic whereby the smears are made uniform. He takes a slide, splits it in two and measures carefully that particular slide. It has to be perfectly plane. One sometimes has to examine perhaps a whole gross of slides before finding one that is suitable. Once that ideal spreader is found it is employed for making all smears, thus doing away with the defects of having an uneven smear. There is another item to be taken into consideration in blood counting and that is the question of rapidity. It is a matter of importance in hospital work where a great many examinations have to be made. To insure that the work is turned out quickly and efficiently the method has to be simple and easily taught to the technician. Any method that will complicate the matter will not be feasible. If as Dr. Nicholson proposes it would be at the same time accurate and easy to carry out, then it would certainly be an advantage to use this method.

Dr. Nicholson (closing).—Differential counts in the counting chamber and cover glass preparation do not vary very much if many cells are counted on the cover glass. There is a much greater variation in counts done by the slide method. Frequently with good technicians the polynuclears will vary 20 per cent. With any method we have the difference in classification. Some will call the lymphocyte with a moderate ring of cytoplasm, a large mononuclear; others will call the young form of polymorph with solid nucleus, that is well indented, a large mononuclear and so forth. If one could find a fluid that would produce an oxidase reaction this difficulty would be largely overcome. The fluid works very well in doing a cell count on spinal fluid. In blood counts done away from the laboratory, the cells show well after the diluted blood remains in the pipette three or four hours. After that time the cytoplasm of the cells becomes rather deeply stained.

ON THE TOXICITY OF TETRAETHYL LEAD AND INORGANIC LEAD SALTS*

By Robert A. Kehoe, M.D., Cincinnati, Ohio

I. INTRODUCTION

In the course of studies on lead poisoning the toxicity of tetraethyl lead has been determined in the case of rabbits, and a comparison has been made with the toxicity of other and commoner lead compounds. The details of the method of study are included herein, and in addition to the data on the toxicity, certain important actions of tetraethyl lead, are presented.

1. It is capable of direct absorption in lethal quantities through the intact skin of rabbits.

2. Its toxicity varies little from that of other and water soluble lead compounds. This fact indicates that its toxicity is a function of the lead, and not of any peculiar qualities characteristic of the compound.

II. MATERIALS

Commercial tetraethyl lead was carefully purified by steam distillation to water white clarity and constant specific gravity. The other chemicals were the best quality, chemically pure materials obtainable.

The animals were healthy rabbits of various ages and sizes, from complete maturity down. They were kept under the best possible laboratory conditions as to food and hygienic conditions.

III. EXPERIMENTAL METHODS

The toxicity of tetraethyl lead was determined on the basis of intravenous, oral, cutaneous and respiratory administration. It was prepared for intravenous injection by dissolving it in sterile cotton-seed oil, so that 1 c.c. represented 0.04 c.c. tetraethyl lead. Injection was made very slowly so as to avoid gross pulmonary embolism.

Cutaneous administration was made after carefully clipping the hair from the belly, without injury to the skin.† The animal was then placed in a hood, with the head directed into the source of air supply, and strong ventilation was employed until the treated area appeared to be dry. This was found to require at least an hour. The animal was then removed from the hood and put into a well ventilated cage for observation.

The material was introduced into the alimentary tract by dropping the

*From the Eichberg Laboratory of Physiology, University of Cincinnati, Cincinnati, Ohio.

Received for publication, September 24, 1926.

†It was found inadvisable to shave the skin, because of the likelihood of slight injuries, and, more important, because moistening and soaping the skin were found to interfere seriously with absorption.

required amount directly into the open mouth of the animal. No difficulty was experienced in causing the animal to swallow the entire dose.

Inhalation studies were made in which air was bubbled through tetraethyl lead and mixed with fresh air, the mixture being passed through tight cages containing animals, in such a manner as to subject the animals to known concentrations of tetraethyl lead vapor. The apparatus for this purpose consisted of a metal box, the metal and glass front of which was fastened on with nuts, a rubber gasket serving to make a tight enclosure. An inlet tube was arranged in the top at one side, and an outlet tube at the opposite side. Air was passed through the cage at the rate of 5 liters per minute, this air being admixed with a varying quantity of air saturated with tetraethyl lead vapor.

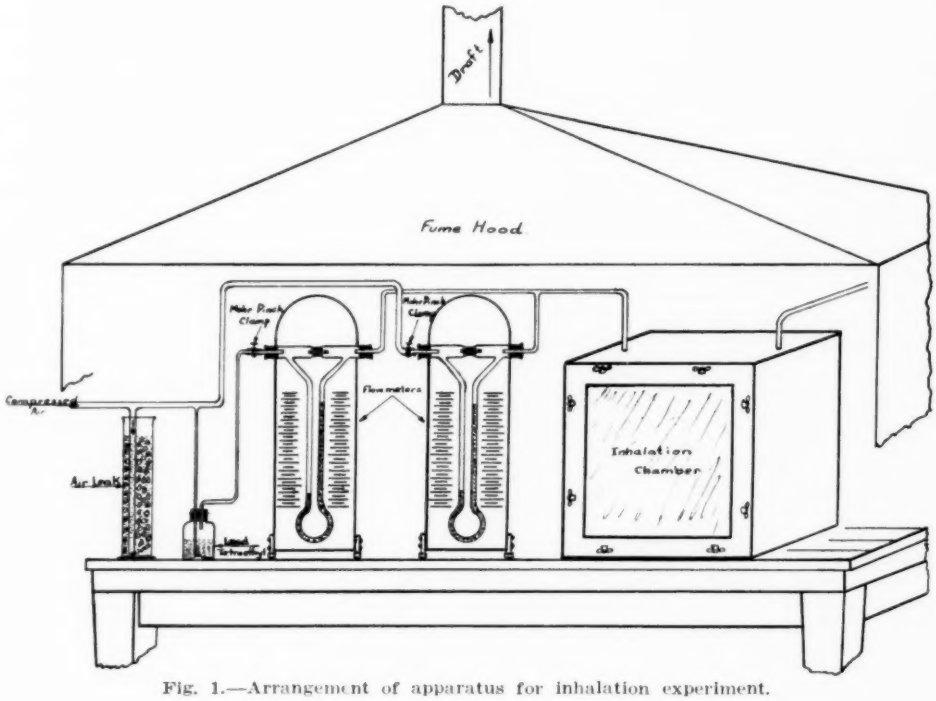


Fig. 1.—Arrangement of apparatus for inhalation experiment.

The volumes of fresh air and of air saturated with tetraethyl lead were measured by means of carefully calibrated flowmeters. Admixture was accomplished by joining the two tubes conveying them with a Y tube, and leading them thus into the cage. Calculations of the lead concentration in the air were made from the previously determined lead content of unit volumes of air saturated with tetraethyl lead at the temperature of the experiment. The apparatus used is illustrated in Fig. 1. The entire operation of this experiment was carried out in a hood with strong forced ventilation. The animals were exposed daily for a period of not to exceed six hours, no food or water being furnished them during the period of exposure.

The toxicity of lead nitrate and lead chloride for rabbits was compared with that of tetraethyl lead, by their administration intravenously.

After treatment in the various ways of the experiment, the animals were observed for the appearance of symptoms. Certain signs and symptoms appeared which came to be recognized as characteristic of acute poisoning.

In case death occurred, the tissues were carefully examined for abnormalities of all kinds. Certain characteristic pathologic changes were found, these being associated at times with other lesions unrelated to those experimentally induced.

From the clinical and postmortem observations it became possible early in the course of the experiments to determine whether death of the animal had been brought about wholly by the poison itself or whether it had been caused in part by previously existing disease or experimental accident.

IV. EXPERIMENTS AND DATA

1. *Intravenous Injection of Tetraethyl Lead.*—A review of the protocols shows the first symptoms appearing in about an hour after the injection of lethal doses of tetraethyl lead. At first there is an increase in respiration, and the animal moves about. Then there is a slowing of the respiratory rate. At this time the animal usually sits quietly as if asleep, arousing occasionally. When he moves, muscular incoordination is observed. The general depression increases and as a terminal manifestation a slight convulsion usually occurs. Death results in from four to twelve hours, dependent upon the dose administered, respiratory paralysis being the immediate cause.

The pathologic findings are set forth in the following necropsy protocol.

Rabbit No. 27.—2.7 kilo, dead ten hours after injection. The lungs are edematous and there are numerous petechial hemorrhages. No infarction is present. The abdominal viscera show general capillary dilatation. The right heart is hugely dilated, the left ventricle being contracted. The pylorus of the stomach is very strongly contracted. The upper small intestine filled with mucoid, watery, yellowish liquid, slightly blood stained in one or two coils. The mucosa is almost completely eroded. The large intestine is unaffected. The kidneys show no gross abnormality. The liver is dark red, the markings are indistinct, and there is general cloudy swelling. The brain is more than normally friable, and there is considerable excess fluid at the base beneath the dura.

The degree of injury to the organs varies with the dosage, and especially with the time which elapses between administration and death. In fact, edema of the brain cannot be detected in an animal which dies quickly. Otherwise the above pathologic findings are constant and characteristic.

Table I shows the results of varying dosages of tetraethyl lead in cottonseed oil (1 c.c. =o= 0.04 c.c. $Pb(Et)_4$) injected intravenously. When the solution is injected pulmonary infarcts are often produced. If injection is made too rapidly, large emboli bring about death quickly. If injection is made very slowly either very small infarcts or none develop, and the animal dies or survives according to the size of the dose.

TABLE I

RABBIT	WEIGHT	DOSAGE OF PB $(ET)_4$	RESULT
18	1.6 kilo	0.004 c.c.	Survived
19	1.9 kilo	0.01 c.c.	Survived
20	1.4 kilo	0.02 c.c.	Survived
24	2.8 kilo	0.033 c.c.	Survived
25	3.0 kilo	0.04 c.c.	Death in five minutes*
27	2.7 kilo	0.04 c.c.	Death approx. 10 hours
28	2.8 kilo	0.04 c.c.	Death approx. 12 hours
33	2.8 kilo	0.03 c.c.	Survived

*Pulmonary embolism.

The above data show that animals of about 3 kilo are uniformly killed by doses of approximately 0.04 c.c. $Pb(Et)_4$ given intravenously. Calculating on the basis of a specific gravity of 1.6591, for $Pb(Et)_4$, the lethal dose per kilo, in terms of Pb is approximately 0.014 gm.

2. *Cutaneous Application of Tetraethyl Lead.*—This method of administration eliminates any factor other than the direct toxic effect of tetraethyl lead. The symptoms, however, are those previously described, plus a striking increase in peristaltic action visible through the abdominal wall, within a few minutes after treatment. There is usually bladder irritation as well, small quantities of urine being voided frequently.

At necropsy the lungs are found to be normal or only moderately congested and the abdominal wall at the site of application is thick with subcutaneous edema. Otherwise the pathologic changes are identical with those produced by intravenous injection.

Table II shows the results of the cutaneous treatment of a series of animals, with pure tetraethyl lead. It may be seen that there is little variation in susceptibility to a single large dose of the compound.

TABLE II

RABBIT	WEIGHT	DOSAGE OF $PB(ET)_4$	RESULT
36	2.4 kilo	0.4 c.c.	Survived
40	2.0 kilo	0.6 c.c.	Survived
69	1.4 kilo	1.0 c.c.	Death in 24 hours
70	1.4 kilo	1.0 c.c.	Death in 24 hours
71	1.3 kilo	1.0 c.c.	Death in 24 hours
124	1.5 kilo	1.0 c.c.	Death in 24 hours
72	1.5 kilo	0.75 c.c.	Survived
73	1.6 kilo	0.75 c.c.	Survived
74	1.6 kilo	0.75 c.c.	Survived
120	2.0 kilo	1.5 c.c.	Death in 24 hours

It is seen from Table II that four animals weighing about 1.5 kilo died when treated with 1 c.c., while three animals of approximately the same weight receiving 0.75 c.c. survived. It is scarcely worth while to sacrifice other animals to attempt a refinement of these results. Considering the unavoidable variation in animals, and the variation in absorbing surface which must occur in such an experiment, the lethal dose for animals of this size is well defined. The above data indicate that the lethal dose of tetraethyl lead for rabbits, when applied cutaneously is approximately 0.7 c.c. per kilo. This calculated in terms of Pb per kilo is equivalent to 0.7 gm.

3. *Oral Administration of Tetraethyl Lead.*—When tetraethyl lead is given by mouth the symptoms and signs of illness and the pathologic lesions which appear are the same as those described above. The process appears to be a little slower, however. None of the animals so treated died in less than two days, and one survived for five days.

Table III shows the results of the experiments.

TABLE III

RABBIT	WEIGHT	DOSAGE OF $PB(ET)_4$	RESULT
37	2.7 kilo	0.04 c.c. (in oil)	No illness
60	2.0 kilo	0.1 c.c. (undiluted)	Ill. Survived
54	1.6 kilo	0.2 c.c. (undiluted)	Died in 5 days
48	1.8 kilo	0.3 c.c. (undiluted)	Died in 2 days

The lethal dose by mouth calculated from the above data is approximately 0.12 c.c. $Pb(Et)_4$ per kilo, or about 0.12 gm. per kilo in terms of Pb.

4. *The Inhalation of Tetraethyl Lead.*—Death of experimental animals from tetraethyl lead poisoning occurs most rapidly if the material is inhaled in high concentration. The saturated vapor of tetraethyl lead may produce death in two hours. The same symptoms of poisoning are seen as have been described, but they develop with surprising suddenness.

The most marked variation in pathology seen in animals treated in this way is in the nasal mucosa, which is red, swollen and covered with an adherent frothy fluid. The trachea and bronchi show no corresponding condition, and the lungs show little edema. In these animals the liver shows much less congestion and cloudy swelling, and the central nervous system is not grossly abnormal. Again the most striking lesion is found in the duodenum, which presents the same characteristic appearance previously described.

Table IV shows the results of studies in which rabbits were exposed to varying concentrations of tetraethyl lead vapor in air.

TABLE IV

RABBIT	WEIGHT	FLOW PER MINUTE THROUGH CAGE		RESULT
		PURE AIR	AIR SAT. WITH $PB(ET)_4$	
3	1.5 kilo	none	5.0 L	Death in 2 hours
6	1.2 kilo	5. L	0.5 L	Death in 6 hours
11	1.6 kilo	5. L	0.25L	Death 3rd day
16	3.3 kilo	5. L	0.20L	Death 3rd day
38	3.4 kilo	5. L	0.15L	Survived after 140 days
32	2.5 kilo	5. L	0.10L	Died 100 days

The above table shows the concentration of tetraethyl lead vapor lethal for rabbits to be about 1 part of saturated vapor of tetraethyl lead in 26 parts air by volume. This is equivalent to 0.175 mg. Pb per liter of air. (1 liter of saturated vapor = 0.00456 gm. Pb at 25°.) The amount of lead which actually passes through the lungs of the animal under conditions which induce death in three days is not capable of accurate determination. Calculating, however, on the approximate value of 20 liters per hour air consumption, the animal was exposed to about 63 milligrams lead as Pb. One such animal contained 32.10 milligrams at death.

5. *Intravenous Administration of Lead Chloride.*—The results obtained by the intravenous injection of lead salts into rabbits are quite variable. There is a considerable variation in susceptibility of the animals, as well as a degree of uncertainty as to the local behavior of the injected solution.

Symptoms of prostration usually appear quickly and the animal may die suddenly within fifteen minutes to an hour after injection. As a general thing some degree of recovery takes place, only to be followed in fatal cases after a variable period of time by weakness, muscular incoordination, drowsiness, and finally death. The latter course has been taken as the indication of general toxicity of the lead compound. The sudden deaths, almost immediately after injection, are the expression of some change occasioned by the intravenous method of administration. That changes in the blood may occur is shown by the frequency with which thrombosis occurs at the site of the injection, and while such injection is under way.

The pathology seen in animals dying after the elapse of several hours following the injection of lead chloride or lead nitrate, is confined almost exclusively to the circulatory system and the kidneys. The heart is enormously dilated (in one case the heart spontaneously ruptured) and there is a dilatation of the capillaries with petechial hemorrhages in the lungs and kidneys. The lungs are edematous, and the kidneys usually show cloudy swelling. The intense duodenal erosion in tetraethyl lead poisoning is absent. There is considerable injection of the intestinal mucosa, however, and a watery diarrhea often gives evidence of intestinal irritation before death.

Table V illustrates the variability of results and points out approximately the lethal dose of lead chloride ($PbCl_2$).

TABLE V

RABBIT	WEIGHT	DOSAGE OF $PBCl_2$ (1 C.C.—5 MG.)	RESULT
43	1.84 kilo	10 mg.	No illness. Survived
44	1.88 kilo	15 mg.	Died in 12 hours
45	1.76 kilo	20 mg.	Survived
154	2.10 kilo	20 mg.	Survived
55	1.64 kilo	25 mg.	Venous thrombosis of ear. Survived
147	2.70 kilo	25 mg.	Died in 41 hours
174	2.00 kilo	25 mg.	Survived
61	1.64 kilo	30 mg.	Diarrhea. Venous thrombosis. Survived
176	2.44 kilo	35 mg.	Survived
178	2.3 kilo	40 mg.	Died in 25 minutes
180	1.72 kilo	40 mg.	Survived
187	1.63 kilo	40 mg.	Survived
193	1.72 kilo	40 mg.	Died in 14 hours
189	1.60 kilo	45 mg.	Died in 24 hours
190	1.62 kilo	45 mg.	Died in 65 hours
195	1.41 kilo	45 mg.	Died in 3 to 4 hours
192	1.77 kilo	45 mg.	Very ill. Survived

There is some indication in the above table that young animals are less susceptible to a single large dose of the lead salt, than are older animals. There are, probably, one or more other factors which influence the result, and which are not fully understood. Although death occurs occasionally from smaller quantities, a dose of 0.040 to 0.045 gm. of $PbCl_2$ is required to kill the

average rabbit of 1.5 to 2.0 kilograms. This is equivalent to from 0.020 gm. to 0.030 gm. per kilo, or in terms of Pb, 0.015 gm. to 0.022 gm. per kilo. Essentially the same results are obtained when $PbNO_3$ is used.

V. DISCUSSION

The foregoing experiments show a close similarity in the toxicity of tetraethyl lead as compared with lead chloride, under conditions in which the amount of lead in the circulatory system is capable of accurate control, namely, intravenous administration. The similarity in toxicity is so close as to warrant the conclusion that toxicity is a function of the common constituent of the two compounds, i.e., lead. The toxicity of heavy metals has long been believed to be due to the coagulation of proteins. Tetraethyl lead has little or no such property, yet it is as toxic as lead salts which have. There is a delay, however, in the appearance of toxicity.* This delay suggests that something happens to tetraethyl lead in the body which brings about a toxicity which is not originally present. There are sufficient evidences that tetraethyl lead breaks down with fair rapidity in the tissues to yield water soluble compounds,† which may be triethyl compounds, or inorganic salts. It is probable that its toxicity is due largely to these decomposition products and that its delayed toxicity as compared with lead salts is due to the rate of decomposition, which does not allow the development of immediately high concentrations of active lead compounds.

However, any advantages to the animal which arise from the relatively nontoxic character of tetraethyl lead as such, are almost counterbalanced by the fat soluble character of the compound, which allows its selective localization in such tissues as will be most affected by it, namely, the nervous tissues. For this reason, tetraethyl lead poisoning is, essentially, a central nervous system intoxication.

VI. SUMMARY

The toxicity of tetraethyl lead has been determined for rabbits for the various methods of administration.

2. Comparison of the toxicity of tetraethyl lead with an inorganic lead salt indicates that its toxicity is a function of its lead content.

3. An explanation of the delayed effect of tetraethyl lead, as compared with salts of lead is offered in that it is suggested that tetraethyl lead owes its toxicity to a decomposition reaction which produces water soluble compounds of lead which are capable of coagulating proteins.

*The delay in toxicity is seen in the skin, where a condition resembling coagulation appears several days after tetraethyl lead has been applied. Also acute symptoms develop slowly even after intravenous administration of many times the lethal dose.

†Evidences of decomposition of tetraethyl lead are seen when it is noted that lead is excreted in the urine in a form from which it is precipitated quantitatively by reagents which do not attack tetraethyl lead.

THE CHLORIDE CONTENT OF CANNED SAUERKRAUT*

By Marian E. Stark, A.B., Madison, Wisc.

IN CONNECTION with an investigation by Dr. William S. Middleton of this hospital into the therapeutic use of sauerkraut in cases of vomiting,[1] a request came to the chemical laboratory to determine the chloride content of the brand of kraut used in the hospital and of one or two others on the local market, for comparison. No figures seemed to be available on this subject. When we had worked out a suitable technic to meet this rather unusual request, we found such a surprising similarity in salt content of the first three samples tested that we became interested in systematically studying enough more brands to determine whether this similarity was accidental or whether canned sauerkraut conforms to a definite standard that can be depended upon for general dietary considerations.

We determined chlorides, therefore, in eleven different cans, which included eight separate brands; in five analyzing both juice and solid, and in the remainder juice only. By this time the very slight variations in figures made it appear evident that a fairly constant "recipe" must be followed by the canners; and since completing our study we have learned that there is, indeed, a federal standard regulating the proportion of salt to be put into this commodity.

In Food Inspection Decision 196, from the United States Department of Agriculture, the following "revised and amended definition and standard for sauerkraut was adopted by the Joint Committee on Definitions and Standards, composed of representatives of the United States Department of Agriculture, the Association of American Dairy, Food and Drug Officials, and the Association of Official Agricultural Chemists, at its meeting July 13 to 17, 1925.

"Sauerkraut is the * * * product * * * obtained by full fermentation * * * of properly prepared cabbage, in the presence of not less than two per cent (2 per cent) nor more than three per cent (3 per cent) of salt. * * *"[2]

In our analyses we actually found the salt content (total chlorides, expressed as NaCl) to range between about 1.5 per cent and 2.2 per cent, with as great variations between two cans from different shipments of the same brands as between different brands. These discrepancies could easily be accounted for by uneven mixing and settling out, in bulk handling of such materials. It was the uniformity, rather than the differences, that impressed us.

The analyses were made by the direct silver titration method of Whitehorn[3] for chloride determinations in blood and urine, adapting the details to the material at hand. Juice and kraut were first separated as well as possible just by thorough draining, with the kraut pressed down under a plate,

*From the Sarah Workman Laboratory of Physiological Chemistry in the State of Wisconsin General Hospital, Madison, Wisconsin.
Received for publication, November 24, 1926.

TABLE I

CHLORIDE CONTENT OF CANNED SAUERKRAUT

Three Brands from Nationally Known Distributors, and Five from More Local (Wisconsin) Packers

BRAND	WT. AND VOL. OF JUICE	WT. OF SOLID	TOTAL WT. OF CONTENTS	PROP. BY WT. OF JUICE	PROP. BY WT. OF SOLID	CHLORIDES, EXPRESSED AS NaCl		
						JUICE		SOLID
						g/100 c.c.	g/100 g.	g/100 g.
F., (a)	168 g. 163 c.c.	615 g.	783 g.	21%	79%	1.865	1.804	1.637
F., (b)	307 g. 298 c.c.	461 g.	768 g.	40%	60%	1.930	1.871	1.774
R.	311 g. 303 c.c.	544 g.	855 g.	36%	64%	1.902	1.855	(a) 1.761 (b) 1.803
B.	323 g. 316 c.c.	592 g.	915 g.	35%	65%	1.799	1.759	(a) 1.605 (b) 1.625
Ri.,* (a)	389 g. 380 c.c.	566 g.	955 g.	41%	59%	2.021	1.974	1.947
Ri., (b)	258 g. 249 c.c.	696 g.	954 g.	27%	73%	2.124	2.053	
S.*	389 g. 380 c.c.	602 g.	991 g.	39%	61%	1.757	1.714	
Sn.	217 g. 210 c.c.	595 g.	812 g.	27%	73%	1.930	1.867	
S.G., (a)	211 g. 205 c.c.	361 g.	572 g.	37%	63%	2.228	2.167	
S.G., (b)	193 g. 188 c.c.	369 g.	562 g.	34%	66%	1.638	1.596	
M.P.	243 g. 238 c.c.	570 g.	813 g.	30%	70%	1.502	1.469	
Averages						1.881	1.830	1.736

*Fading end-point encountered in titration. See text.

as would be done in kitchen handling. The juice was centrifuged at about 3000 R. P. M. to remove gross débris, and duplicate portions diluted to contain a chloride concentration found, by trial and error, to be in the range expected for blood filtrates. The titration as for the latter could then be employed unchanged. The solid kraut was extracted with water as thoroughly as possible. Weighed portions were first ground to a pulp in a mortar with a little water, and the mass washed repeatedly, the washings being collected through a filter, with the aid of gentle suction into a volumetric flask of appropriate capacity for final dilution. The same dilution could be used for all the samples, both juice and solid, throughout. Preliminary tests had indicated that not enough protein material was present to interfere with the titration.

Each figure for chlorides in the table represents the average of at least two titrations. Duplicates agreed in all cases but one within a variation of 0 to 0.02 c.c., which represents a maximum deviation of 0.7 per cent. By duplicates are meant determinations on separate dilutions, for the juice; but for the solid, titrations of duplicate aliquots of the diluted washings from the same original weighed sample. In two cases, in addition, separate weighed samples were determined from the same can, and these agreed within 2.3 per cent and 1.2 per cent, respectively, of each other. The inherent possibilities for error in the sampling of the solid materials are obvious.

End-points in the titration were as sharp and definite as the method usually gives, with the exception of the two brands noted with asterisks in the table. In the case of "Ri., a," excessive turbidity of the juice, the odor, and the degree of softness of the cabbage, pointed to either a greater thoroughness of fermentation, or an unusual strain of fermenting organisms. It was chiefly the juice of the first can of this brand that was troublesome to titrate, but the average of eight different determinations (maximum discrepancy 2.7 per cent) happened to give a figure very similar to that obtained, with no such difficulty, on a second can of the same brand from a different lot. With the other can marked, there was a slight tendency to fading end-point, but four duplicates agreed very closely.

The figures in the Table for the amounts of juice and solid and their relative proportions in each can are presented as of incidental interest, and expressed in round numbers.

SUMMARY

Analyses of the salt content of canned sauerkraut gave values as follows for total chlorides, expressed as NaCl:

For juice (eleven cans, including eight brands): In grams per 100 c.c.: average, *1.881;* maximum, 2.228; minimum, 1.502. In grams per 100 grams (per cent): average, *1.830;* maximum, 2.167; minimum, 1.469.

For solid kraut (seven samplings from five cans, including four brands): In grams per 100 grams (per cent): average, *1.736;* maximum, 1.947; minimum, 1.605.

REFERENCES

[1]Middleton, Wm. S.: Wisconsin Med. Jour., November, 1926, xxv, 554.
[2]Food Inspection Decision 196, from the Dept. of Agriculture, Pamphlet 58270-25, from the U. S. Government Printing Office, 1925.
[3]Whitehorn, J. C.: J. Biol. Chem., 1921, xlv, 449.

A NOTE UPON COMPLEMENT FIXATION IN TUBERCULOSIS*

By Isamu Ogawa, M.D., Mukden, Japan

THE complement-fixation reactions reported upon in this communication were conducted according to the technic of the new Kolmer complement-fixation test for syphilis.[1] The qualitative method was employed using 0.1 c.c. of serum and 0.3 c.c. of pleural fluid (both heated at 55° C. for fifteen minutes) with several different antigens. Each antigen was titrated for anti-complementary activity and used in an amount corresponding to one-third of their unit, the technic employed in all titrations and complement-fixation tests being exactly as described by Kolmer, including an antisheep hemolytic system with titration of hemolysin and complement, a primary incubation of eighteen hours at 6 to 8° C. followed by ten minutes in a water-bath, etc.

*From the Japanese Red Cross Hospital, Mukden.
Received for publication, October 12, 1926.

The first part of the study was conducted in Berlin (Prof. Heyman's clinic) with Petroff and Besredka antigens, and it may be well to note in passing that the German literature contains but few references to the complement-fixation reaction in tuberculosis although that by Rabinowitsch-Kempner[2] would appear to express the consensus of opinion, namely, that the tuberculosis complement fixation is a practical and specific means for diagnosis in that a positive reaction indicates the presence of an active lesion while a negative reaction may occur in healed and clinically latent cases.

The second and larger part of the investigation was conducted in the Japanese Red Cross Hospital of Mukden employing the Kolmer and Besredka antigens.

The serum of experimentally infected guinea pigs was used in addition to a large number of serums from tuberculous and nontuberculous human subjects. As stated three antigens were employed, namely, those prepared after the methods of Besredka, Petroff and Kolmer and one of the interesting phases of the study was a comparison of them.

RESULTS OBSERVED IN BERLIN

Guinea pigs were inoculated with various acid-fast bacilli including different strains of *B. tuberculosis* and saprophytes. All were killed in twenty to seventy days and the serum tested. Petroff and Besredka antigens prepared of human, bovine and fowl strains of tubercle bacilli yielded a high percentage of positive reactions; Kolmer antigen was not available for these tests. It was especially interesting to note, however, that animals infected with some of the acid-fast saprophytes gave no complement fixation with tuberculosis antigen.

Cases of tuberculosis presenting undoubted clinical evidences along with bacilli in the sputum were classified arbitrarily in first second and third classes according to the tuberculous state, the third class being the most advanced. Out of 125 such cases belonging to all three classes, from 60 to 90 per cent yielded a positive reaction with Petroff's antigen:

First class: 19 tested; 17 or 90 per cent positive.
Second class: 54 tested; 43 or 80 per cent positive.
Third class: 52 tested; 22 or 61 per cent positive.

Ninety-six of these were also tested with Besredka's antigen with 67 to 85 per cent positive reactions as follows:

First class: 14 tested; 12 or 85 per cent positive.
Second class: 45 tested; 37 or 82 per cent positive.
Third class: 37 tested; 25 or 67 per cent positive.

It will be observed, therefore, that in this group positive reactions occurred in 42 to 90 per cent of cases, the highest percentage of negative reactions being observed with the sera of those cases presenting the most advanced stages of tuberculosis and rapidly approaching death. Also the Petroff antigen proved somewhat superior to that of Besredka.

In a second group of 56 persons presenting no clinical evidences of tuberculosis, the sera of nine yielded positive reactions with the Petroff antigen.

But five of these persons were known to be syphilitic and the positive reactions were in all probability Wassermann reactions with the antigen furnished by the lipoids of the bacilli. The Besredka antigen also yielded positive reactions with the sera of syphilitic patients and appeared to give this cross-complement-fixation reaction more frequently and more strongly than the Petroff antigen.

Of 57 patients regarded clinically as in *early* tuberculosis and without expectoration of bacilli, 19, or 33 per cent, reacted positively with the Petroff antigen and 21 or 37 per cent with the Besredka antigen. In other words fully one-third reacted negatively and this is unfortunate, since the complement-fixation test may fail, therefore, to be of material aid in the diagnosis of early and clinically obscure and difficult cases. It is to be emphasized, however, that some of those examined may not have been truly tuberculous.

RESULTS OBSERVED IN MUKDEN

Of 27 patients of advanced tuberculosis expectorating bacilli, 26, or 97 per cent, yielded positive reactions with the Kolmer antigen. The one negative case was so far advanced that death resulted seventeen days after the test was made. The sera of eleven cases of this same series were tested with the Besredka antigen and 6, or 55 per cent reacted positively.

Of 111 cases regarded as tuberculous but without expectoration of bacilli, 99, or 89 per cent, reacted positively with the Kolmer antigen; 22 of these were tested with the Besredka antigen and 10, or 45 per cent, reacted positively.

Of the pleural fluids from 50 cases of tuberculous pleuritis tested with the Kolmer antigen, 38 or 76 per cent, yielded positive reactions. Of 68 fluids tested with the Besredka antigen, 15, or 22 per cent reacted positively.

The Kolmer antigen, therefore, yielded the highest percentage of positive reactions, but that some of these may have been due to the acquisition of anti-complementary properties on the part of the antigen in transit from the laboratory in Philadelphia, is indicated by the occurrence of as high as 37 per cent of doubtfully or weakly-positive reactions with the sera of persons regarded clinically as nontuberculous; in this same group the Besredka antigen yielded about 5 per cent positive reactions.

In a group of 79 cases of syphilis with positive Wassermann reactions, the Kolmer and Besredka antigens yielded from 20 to 66 per cent positive reactions; some of these persons were probably tuberculous as well but I feel quite sure that both antigens are capable of yielding cross-complement-fixation reactions with syphilis antibody.

REFERENCES

[1]Am. Jour. Syph., 1922, vi, 82.
[2]Deutsch. Med. Wchnschr., 1922, xlviii, 379.

IMMUNIZATION OF RATS AGAINST PNEUMOCOCCUS BY FEEDING THE ACID-KILLED GERMS AND THE INFLUENCE OF THE AGE OF THE ANIMAL THEREON*

By Victor Ross, Ph.D., Bloomfield, N. J.

INTRODUCTION

OUR earlier demonstration that albino rats could be immunized against intraperitoneal injections of virulent pneumococci by feeding tissues of other animals killed by the same organism,[1] or by feeding the living germ,[2] has naturally suggested the question whether dead germs would function in the same manner. The result of two experiments in which the germs, before being used, were heated at 80° C. for two hours has already been reported and showed that little or no protection resulted against an injection of living pneumococci. At that time we mentioned that we would attempt the use of HCl acid-killed germs as an immunizing agent. Several experiments, performed largely on rats and mice, in which pneumococci killed in this manner were employed, have since been completed and form the basis of this report. The influence of the age of the animal was also studied because of its importance in any practical application of the results obtained to humans.

Three experiments were done on rats. In the first two the purpose was to learn whether pneumococci killed by hydrochloric acid would serve as well as pneumonic tissue and live germs. In the third, the influence of the age of the rat was studied, the use of acid-killed germs having been found to be effective.

METHODS

Pneumococcus, Type I, was used throughout and was grown either in beef extract or beef heart infusion with or without 0.5 per cent glucose. Sufficient normal HCl acid was added to such cultures to make the final concentration N/15 or N/20 HCl. After two hours, at room temperature, the whole was centrifuged, the germs suspended in water or in 0.1 per cent gelatin solution and cracker meal added and stirred. In those cases where heat-killed germs were used, they were grown on beef heart infusion, without glucose, and after centrifugation and suspension in gelatin solution were heated at 90° C. for two hours, the cracker meal was added and the whole fed. Fresh cultures were used daily. The germs from 50 c.c. culture were fed to each rat daily for an average period of three weeks.

Comparison of the resistance of treated and control animals was made by injecting intraperitoneally various amounts of the virulent organism, type I, in a constant volume of 0.20 c.c. Controls were always injected at the same time as treated animals so that the virulence of the culture was known for the day on which the test was made.

*From the Plaut Research Laboratory, Lehn and Fink, Inc., Bloomfield, N. J.
Received for publication Nov. 3, 1926.

RESULTS AND DISCUSSION

Table I gives the results of the experiments in which rats were fed pneumococci killed by HCl acid. In it are given the date of the experiment, the number of the rat, whether it is a control or treated animal, its weight, the dose injected to test the animal's resistance and the result. It is evident, from the rapid death produced in the controls on March 21 by 10^{-5} c.c., that a smaller quantity would also have been fatal. The two experimental rats 343 and 329 both died, due to an overdose. On the 30th the culture was weak. On April 2, $2x10^{-4}$ c.c. was fatal for the control, but the treated rat survived this amount. On the 3rd 10^{-6} c.c. killed a control; of two immunized rats, each receiving 10^{-3} c.c., one lived. On the 5th 10^{-6} c.c. was again fatal for a control; two of four treated animals survived 10^{-3} c.c., two died of this quantity. On the 7th, $2x10^{-6}$ c.c. killed a control; two experimental rats died of $2x10^{-4}$ c.c.; three of $2x10^{-3}$ c.c., and one of $2x10^{-2}$ c.c. One survived $2x10^{-4}$ c.c. and another $2x10^{-5}$ c.c. On the 9th, 12th and 17th, 10^{-7} c.c. killed controls and on the 15th, 10^{-8} c.c. was fatal. On the 15th, all the experimental animals died, having received at least 10,000 and 100,000 times the fatal dose which was more than they could tolerate. On the other days, in spite of some irregularity in the results it is apparent that the immunized rats tolerate in general 1,000 times as large a dose as the untreated animals.

The rats, data for which are given from May 5 to June 2 form a group immunized at a different time. On May 5, 1,000 and 10,000 times the fatal dose did not kill the immunized animals. On the 10th, 10^{-7} c.c. killed a control rat whereas 10^{-4} c.c. failed to kill either of two experimentals. 10^{-3} c.c. was not tried. On the 14th, a control rat succumbed to 10^{-8} c.c., another survived this amount. Three treated rats survived 10^{-4} c.c. and two died of this dose; two survived 10^{-5} c.c. The remaining tests on the 19th, 21st, 24th, 26th and 28th of May and on June 2 all demonstrate that a decided degree of immunity exists among the rats fed with acid-killed germs. The results in this group of rats are more regular and show a higher degree of immunity than those of the preceding one.

In Table I, beginning with July 20, are given the results of a similar experiment in which the additional influence of age of the animal was tested. Although the two preceding experiments were done with rats varying in weight from 112 to 175 gm. in the first and from 45 to 147 gm. in the second, thus covering the approximate ages of one to three months, they had been performed without an attempt to observe any influence of this factor. The present experiment was carried out primarily with this object in view, feeding acid-killed organisms which were now known to be as effective as living germs. One group of rats weighed between 62 and 70 gm. when feeding of germs was started, the other weighed between 263 and 305 gm. at the time. Although the exact ages of these groups of animals were unknown, their weights indicated that they were respectively about two months and twelve months. The latter age represents about one-third of the life span of the rat. Both groups were treated identically with regard to food and quantity of germs fed. The virulence of the culture was tested on July 20 and 22; 10^{-7}

TABLE I

PROTECTION AGAINST INTRAPERITONEAL INJECTION OF PNEUMOCOCCUS, TYPE I, AFFORDED RATS BY FEEDING THE HCl ACID-KILLED GERM. C = CONTROL, E = TREATED, D = DIED –DAYS, S = SURVIVED

DATE 1926	RAT NO.	WT. GM.	DOSE C.C.	RESULT	DATE 1926	RAT NO.	WT. GM.	DOSE C.C.	RESULT
3/21	C	180	10^{-5}	D1	4/15	C	221	10^{-5}	D2
	C	175	10^{-5}	D2		C	196	10^{-6}	D2
	343E	185	$2x10^{-3}$	D2		C	180	10^{-7}	D2
	329E	165	10^{-4}	D2		C	171	10^{-8}	D3
						315E	210	10^{-3}	D1
3/30	C	185	10^{-4}	S		321E	215	10^{-3}	D1
	C	180	10^{-5}	S		344E	230	10^{-3}	D2
	342E	167	10^{-4}	S		352E	230	10^{-3}	D2
	351E	165	10^{-5}	S		327E	205	10^{-4}	D2
						325E	206	10^{-4}	D2
4/2	C	205	$2x10^{-4}$	D2		337E	200	10^{-4}	D2
	314E	205	$2x10^{-4}$	S		347E	195	10^{-4}	D2
4/3	C	240	10^{-4}	D2	4/17	C	255	10^{-5}	D2
	C	203	10^{-5}	D2		C	185	10^{-6}	D2
	C	185	10^{-6}	D2		C	170	10^{-7}	D2
	313E	180	10^{-3}	D2		C	167	10^{-8}	S
	310E	213	10^{-3}	S		C	163	10^{-9}	S
						317E	255	10^{-3}	D2
4/5	C	217	10^{-4}	D2		316E	239	10^{-4}	D5
	C	188	10^{-5}	D2		320E	233	10^{-4}	S
	C	210	10^{-6}	D2		333E	205	10^{-4}	S
	349E	220	10^{-3}	D1		331E	221	10^{-4}	D2
	335E	190	10^{-3}	D2		324E	206	10^{-5}	S
	318E	195	10^{-3}	S		A307E	233	10^{-5}	S
	A305E	207	10^{-3}	S		328E	200	10^{-5}	S
						346E	190	10^{-5}	D3
4/7	C	222	$2x10^{-4}$	D2		309E	195	10^{-6}	S
	C	220	$2x10^{-5}$	D2		340E	175	10^{-6}	D2
	C	203	$2x10^{-6}$	D2					
	324E	240	$2x10^{-2}$	D1	5/5	C	182	10^{-5}	D5
	A303E	220	$2x10^{-3}$	D1		C	165	10^{-6}	D5
	319E	240	$2x10^{-3}$	D1		C	143	10^{-7}	D4
	323E	210	$2x10^{-3}$	D2		C	143	10^{-8}	D6
	338E	230	$2x10^{-4}$	D1		373E	179	10^{-4}	S
	339E	224	$2x10^{-4}$	S		371E	173	10^{-5}	S
	330E	228	$2x10^{-4}$	D2		369E	178	10^{-5}	S
	348E	208	$2x10^{-5}$	S		374E	143	10^{-6}	S
4/9	C	235	10^{-5}	D2	5/6	C	150	$2x10^{-5}$	D2
	C	203	10^{-6}	D2		C	150	$2x10^{-7}$	D3
	C	185	10^{-7}	D3					
	C	174	10^{-8}	S	5/10	C	195	10^{-5}	D2
	A308E	208	10^{-3}	D2		C	166	10^{-6}	D3
	311E	200	10^{-4}	S		C	160	10^{-7}	D2
	345E	225	10^{-4}	S		C	155	10^{-8}	S
	A306E	201	10^{-5}	S		370E	192	10^{-4}	S
	350E	197	10^{-5}	S		356E	195	10^{-4}	S
	332E	180	10^{-5}	S		355E	165	10^{-5}	S
						376E	149	10^{-5}	S
4/12	C	232	10^{-5}	D2					
	C	202	10^{-6}	D2	5/14	C	220	10^{-5}	D2
	C	177	10^{-7}	D2		C	177	10^{-5}	D3
	C	162	10^{-8}	S		C	185	10^{-6}	S
	326E	242	10^{-3}	S		C	143	10^{-6}	D4
	336E	195	10^{-3}	D2		C	163	10^{-7}	D3
	A304E	160	10^{-4}	D2		C	143	10^{-7}	D4
	312E	175	10^{-4}	S		C	160	10^{-8}	D5
	334E	175	10^{-4}	D2		C	140	10^{-8}	S
	341E	176	10^{-4}	S		C	155	10^{-9}	S

TABLE I—CONT'D

DATE 1926	RAT NO.	WT. GM.	DOSE C.C.	RESULT
5/14	364E	173	10^{-3}	D4
	402E	166	10^{-4}	S
	363E	165	10^{-4}	S
	401E	142	10^{-4}	S
	354E	153	10^{-4}	D3
	362E	144	10^{-4}	D2
	398E	190	10^{-5}	S
	399E	170	10^{-5}	S
5/19	C	223	10^{-5}	D5
	C	178	10^{-6}	D2
	C	148	10^{-7}	D3
	C	146	10^{-8}	S
	353E	178	10^{-4}	D6
	389E	170	10^{-4}	S
	390E	142	10^{-5}	S
	380E	153	10^{-5}	S
5/21	C	160	10^{-5}	D3
	C	158	10^{-6}	D3
	C	136	10^{-7}	D4
	C	135	10^{-8}	D4
	358E	183	$2x10^{-3}$	D4
	365E	146	10^{-4}	D4
	360E	138	10^{-4}	S
	400E	138	10^{-5}	S
5/24	C	200	10^{-4}	D4
	C	173	10^{-5}	S
	C	148	10^{-6}	D6
	C	146	10^{-7}	S
	C	141	10^{-8}	S
	379E	173	$2x10^{-3}$	D5
	368E	148	10^{-4}	S
	388E	147	10^{-4}	S
	396E	145	10^{-5}	S
	395E	147	10^{-5}	S
	381E	144	10^{-5}	S
5/26	C	225	$2x10^{-3}$	D4
	C	145	$2x10^{-3}$	D4
	C	185	10^{-4}	D4
	C	150	10^{-5}	D4
	C	138	10^{-6}	D3
	C	137	10^{-7}	D3
	C	132	10^{-8}	S
	382E	158	10^{-4}	S
	366E	158	10^{-4}	D2
	394E	145	10^{-4}	S
	378E	144	10^{-5}	S
	384E	143	10^{-5}	D6
	383E	142	10^{-5}	S
5/28	C	136	10^{-5}	D2
	C	129	10^{-6}	D5
	C	128	10^{-7}	D2
	C	121	10^{-8}	D3
	385E	123	10^{-4}	S
	E	126	10^{-4}	D3
	386E	121	10^{-5}	D2
	387E	119	10^{-5}	S
6/2	C	185	10^{-5}	D5
	C	138	10^{-6}	S
	C	132	10^{-7}	D3
	C	130	10^{-8}	D6
	375E	161	10^{-4}	S
	361E	159	10^{-4}	S
	372E	142	10^{-5}	D4
7/20	C	125	10^{-8}	D6
	C	125	10^{-7}	S
	C	125	10^{-6}	D3
7/22	C	115	10^{-7}	D2
	C	135	10^{-6}	D3
	C	285	10^{-5}	D2
	C	310	10^{-4}	D2
	C	335	$2x10^{-3}$	D4
7/23	C	124	10^{-6}	D2
	C	129	10^{-5}	D2
	C	252	10^{-6}	D2
	C	304	10^{-5}	D2
	419E	116	10^{-6}	S
	417E	124	10^{-5}	S
	445E	285	10^{-6}	S
	450E	298	10^{-5}	S
7/26	C	117	10^{-6}	D2
	C	120	10^{-5}	D2
	C	295	10^{-6}	D3
	C	305	10^{-5}	D2
	415E	110	10^{-5}	S
	407E	110	10^{-5}	D7
	428E	320	10^{-5}	S
	435E	325	10^{-4}	D2
7/27	C	133	10^{-8}	S
	C	138	10^{-7}	D2
	C	146	10^{-6}	D2
	C	153	10^{-5}	D2
	C	312	10^{-7}	D2
	C	315	10^{-6}	D2
	C	318	10^{-5}	D2
	427E	129	10^{-5}	S
	420E	131	10^{-5}	S
	411E	149	10^{-4}	S
	418E	151	10^{-4}	S
	431E	310	10^{-5}	S
	429E	314	10^{-5}	S
	436E	322	10^{-4}	S
	451E	323	10^{-4}	S
7/30	C	154	10^{-8}	S
	C	156	10^{-7}	D2
	C	158	10^{-6}	D2
	C	165	10^{-5}	D2
	C	300	10^{-8}	D2
	C	300	10^{-7}	D2
	C	305	10^{-6}	D2
	C	316	10^{-5}	D2
	404E	160	10^{-5}	S
	410E	157	10^{-5}	S
	421E	168	10^{-4}	S
	416E	171	10^{-4}	S
	444E	300	10^{-5}	S
	448E	301	10^{-4}	S

TABLE I—CONT'D

DATE 1926	RAT NO.	WT. GM.	DOSE C.C.	RESULT	DATE 1926	RAT NO.	WT. GM.	DOSE C.C.	RESULT
7/30	430E	308	10^{-4}	D2		C	156	10^{-7}	D4
	452E	312	$2x10^{-3}$	S		C	156	10^{-6}	D2
						C	156	10^{-5}	D1
7/31	C	153	10^{-8}	D2		C	273	10^{-10}	S
	C	154	10^{-7}	S		C	296	10^{-9}	S
	C	154	10^{-6}	D2		C	305	10^{-8}	D4
	C	157	10^{-5}	D2		C	311	10^{-7}	D6
	C	305	10^{-9}	D2		C	335	10^{-6}	D3
	C	303	10^{-8}	D3		C	335	10^{-5}	D2
	C	309	10^{-7}	D2		409E	170	10^{-5}	S
	C	320	10^{-6}	D2		403E	168	10^{-5}	S
	C	325	10^{-5}	D2		412E	171	10^{-4}	S
	405E	108	10^{-6}	S		416E	173	10^{-4}	S
	413E	145	10^{-5}	S		433E	270	10^{-6}	S
	425E	163	10^{-4}	S		437E	322	10^{-5}	S
	422E	169	$2x10^{-3}$	D2		446E	255	10^{-6}	S
	432E	298	10^{-5}	D2		443E	292	10^{-5}	S
	442E	311	10^{-4}	S		439E	323	10^{-5}	S
	449E	305	10^{-4}	D2		440E	323	10^{-4}	S
	447E	319	$2x10^{-3}$	D1		438E	325	10^{-4}	S
						441E	333	$2x10^{-3}$	D2
8/3	C	153	10^{-9}	S					
	C	153	10^{-8}	S					

c.c. killed a small rat and 10^{-5} c.c. a large one. Subsequently it was found, as the figures indicate, that a much smaller dose killed large control rats. On the 23rd of July, small and large controls died of 10^{-6} c.c. whereas the treated rats survived 10^{-5} c.c. On the 26th, one small treated rat survived 10^{-5} c.c., another died of this amount. It was the only rat of this group which failed to tolerate this quantity. Of two large rats tested, one survived 10^{-5} c.c., the other succumbed to 10^{-4} c.c. On the same date 10^{-6} c.c. killed both small and large controls. On the 27th, 10^{-7} c.c. killed both small and large control rats, whereas eight small and large rats fed the germs survived doses of 10^{-5} c.c. and 10^{-4} c.c. On the 30th, a small control survived 10^{-8} c.c., another died at 10^{-7} c.c., whereas large controls died of both these amounts. Small experimental rats tolerated 10^{-4} c.c. and 10^{-5} c.c. One large experimental lived after being injected with 10^{-5} c.c., another after 10^{-4} c.c.; a third died of the latter quantity and a fourth survived $2x10^{-3}$ c.c. This last animal therefore withstood at least 200,000 times a dose fatal for a control. On the 31st, a small experimental rat lived after receiving 10^{-4} c.c. but another died of $2x10^{-3}$ c.c., 10^{-5} c.c., 10^{-6} c.c., and 10^{-8} c.c., similarly killed small controls; a control injected with 10^{-7} c.c. survived. Of the large experimental rats three succumbed to $2x10^{-3}$ c.c., 10^{-4} c.c., and 10^{-5} c.c., respectively; one receiving 10^{-4} c.c. survived. On this day 10^{-9} c.c. killed a large control. The unusual virulence of the culture explains the failure of a larger proportion of treated rats to survive. On August 3, 10^{-7} c.c. proved fatal for a small control, 10^{-8} c.c. for a large one. A large experimental rat died of $2x10^{-3}$ c.c., eleven other rats receiving smaller doses all survived. There is thus consistent evidence showing that feeding the HCl acid-killed pneumococci protects rats whether young or adult, against subsequent intraperitoneal injection of the same living organism. The data show clearly that the adult rats are protected by this

treatment fully as well as the young ones. It is perhaps, therefore, not unsafe to assume that rats older than twelve months are also susceptible to immunization by this process, if not to an equal degree, at least partially.

It is of interest to note the relative susceptibility of small and large untreated rats to intraperitoneal injection of the pneumococcus. On July 23 and 26 both adult and young animals succumbed to 10^{-6} c.c. No smaller quantities were employed. On the 27th, 10^{-7} c.c. was likewise fatal to both kinds; 10^{-8} was not used on a large animal but failed to kill a small one. On the 30th, 10^{-8} c.c. again failed to kill a young rat but did kill an adult animal. On the following day this dose proved fatal for both kinds. Also 10^{-9} c.c. killed a large control. We do not know whether a small rat would have died of this amount because it was not used. One small control did, however, survive 10^{-7} c.c. On August 3, an adult control succumbed to 10^{-8} c.c., whereas a young one survived. Although these figures are too few to warrant drawing a conclusion, they indicate that adult rats approximately one year old are less resistant to Type I pneumococcus than rats about two months old. There appears to be a similar relation in regard to toxic chemicals.[3]

Three separate experiments were done on mice with irregular results. It is at present impossible to explain the irregularity in the response of mice to the feeding process. The quantities fed were larger than required if calculated on the basis of weight of animal, using the rat as standard.

In addition to the considerable difference obtaining between rats and mice in the regularity with which they can be immunized by this procedure, there is another worthy of mention. Among the several hundred rats which have been fed either *living* pneumococci or the tissues of animals killed by intraperitoneal injection of this organism, only occasionally has one died, and never has the organism been recovered from the heart's blood of the dead rat, although efforts were made to do so. In October of 1925 when rats were being immunized by feeding the infected tissue an experiment was started in which mice were similarly treated, using mouse tissue. Of 31 such mice, 30 succumbed within a period of two weeks. Of 30 control mice fed on normal tissues of healthy mice sacrificed for the purpose 15 died in the same length of time. This shows that although mice fed raw tissue die, mice fed on tissue containing pneumococci die in even larger numbers. Substantiation of these results was obtained in the experiments discussed above in which living germs alone were fed without tissue. Of 26 grown mice fed such organisms six died.

We have done only a few experiments with rabbits which yielded negative results.

It can now be stated that the extent of the immunity conferred by using acid-killed organisms is as great as when pneumonic tissue or living germs are employed and that adult rats are as fully protected as young ones. It has previously been shown that the minimum duration of the protection afforded by feeding pneumonic tissue is four months[4] at which time it is as marked as at the beginning. To a lesser extent it may last considerably longer. We do not yet know whether an equal duration of protection is procured when dead pneumococci are used. The use of acid-killed organisms, however, simplifies

any possible application of the procedure to human beings, which is the ultimate aim of the work. The similarities existing between the rat and man encourage the belief that the latter may also be immunized by feeding the pneumococcus.

Our present experiments are being made with monkeys.

CONCLUSIONS

1. Rats can consistently be immunized against intraperitoneal injection of pneumococcus, Type I, by feeding the acid-killed organism.

2. Adult rats are as well protected by this procedure as young ones.

REFERENCES

[1]Ross, Victor: Jour. Immunol., 1926, xii, 219-229.
[2]Ross, Victor: Jour. Immunol., 1926, xii, 237-249.
[3]Donaldson, H. H.: The Rat, Baltimore, 1924, ed. 2, p. 328.
[4]Ross, Victor: Jour. Immunol., 1926, xii, 323-330.

THE CHEMOTHERAPY OF STREPTOCOCCEMIA*

By Robert A. Kilduffe, A.M., M.D., Atlantic City, N. J.

IN RECENT years a great deal of study has been directed toward the development of an efficient method for the treatment of blood stream infections by the intravenous administration of various dye compounds of varying degrees of bacteriostatic and bactericidal activity, among the more prominent of which is mercurochrome-220 soluble, a product formed by the combination of mercury and a fluorescent dye.

The literature abounds in conflicting reports as to the efficiency of this product. While, on the one hand, startling and dramatic instances are reported suggesting marked and rapid sterilization of the blood stream by this drug, on the other hand, equally significant reports are available in direct conflict with such conclusions.

Walker,[1] for example, in an investigation of the bactericidal properties of freshly defibrinated blood to which mercurochrome was added in varying concentrations, found no increase in bactericidal activity toward the colon bacillus in concentrations of mercurochrome as high as 1:400; with concentrations of 1:200 the bactericidal activity of the blood was destroyed. He also found that in mercurochrome-blood mixtures of 1:400, staphylococci and streptococci were not only not destroyed but that these organisms grew more luxuriantly than in plain blood. The explanation of this, according to Walker, lies in the injurious action of mercurochrome on the leucocytes.

The occurrence of numerous and directly contradictory reports of both clinical and experimental investigations has left the subject in a somewhat

*Read before the monthly meeting of the Atlantic City Hospital Staff, Friday, November 19, 1926.
Received for publication Nov. 27, 1926.

confused position and indicates the necessity for the collection and analysis of detailed and minute data of both kinds.

It is apparent that clinical experimentation can seldom be conceived, regulated, or followed out in strict accordance with preconceived plans as can be done in laboratory experiments, the various features of which are under more or less rigid control.

It is possible, however, by the careful record of clinical minutiae to accumulate data capable of relatively accurate evaluation, and it may be contended that one case in which all the minutiae possible are painstakingly recorded may ultimately prove more informative than a series the account of which permits of *post hoc ergo propter hoc* conclusions.

The present report is an endeavor to furnish such data.

Until their enforced modification by the information thus far accumulated, two conceptions have dominated the chemotherapy of infection: the conception of a therapia sterilisans magna, and that of a special chemical affinity of the drug and the organism.

There is clinical evidence to support the former hope and experimental evidence to contradict; and some, at least, of the opposed experiences are explainable on the basis of uncontrolled observations or unsupported assumptions and, particularly, as Churchman[2] points out, by a lack of definition as to what constitutes a bacteriemia.

Certain facts seem assured:

1. Under certain conditions, and for certain bacteria, mercurochrome may be bactericidal and, still under certain conditions, such bactericidal property may be exhibited in the blood stream.

2. For still other organisms mercurochrome may be bacteriostatic, capable of inhibiting their growth without necessarily encompassing their destruction.

3. Chemotherapy, as Kolmer has said, cannot be dissociated from the possibility of chemopathology which may be cumulative, dangerous, and even fatal.

While Young, Scott and Hill[3] have reported the ingestion of 900 mg. of mercurochrome daily for ten days without injury, and Hill and Bidgood[4] that, unless the dose is at least 10 mg. per kilo, renal damage does not follow the intravenous administration of mercurochrome, St. George[5] reports definite autopsy evidence of mercury poisoning in five mercurochrome-treated cases, mercury being recovered from the viscera in amounts larger than those seen in bichloride poisoning. The largest amount given was 0.9 gm., the smallest 0.1 gm.

Corper[6] likewise has called attention to the pathologic alteration of tissues directly exposed to the action of mercurochrome.

It is apparent, therefore, that this, and comparable preparations suggested as chemotherapeutic agents, cannot be expected to produce inevitably a therapia sterilisans magna and that they must be used with care and due regard for the cumulative and even dangerous chemopathology which may result.

The belief may be expressed that the true evolution of mercurochrome intravenous therapy of bacteriemia has been made difficult by the predominant importance given to its *bactericidal* activity and that attempts to achieve a therapia sterilisans magna have overshadowed all other possibilities and dominated its clinical, as well as, to a somewhat lesser extent, its experimental use.

The relatively large and frequent doses of solutions seldom weaker than 1 per cent and, when the concentration was lower, the use of larger quantities to compensate for the lessened strength of the solution, is apparent from the cases reported which lend support to this belief.

Nearly always efforts have been focused upon the massive and complete sterilization of the blood as the main, if not the sole, issue.

It can be debated if this is always the most important—or even the most desirable—consummation to be sought, for, as Churchman[2] has pointed out, septicemia—bacteriemia—may include a variety of conditions, the gravity of which is influenced by a variety of factors.

Among these are: (a) the virulence of the invading organisms; (b) the accessibility to treatment of their source, the focus of infection; (c) the resistance of the patient; and (d) the site and gravity of the secondary lesions in various tissues or organs resulting from bacterial localization.

In every bacteriemia the bacteria enter the blood stream from some initial focus and are removed from the blood stream by the defensive mechanism, namely, the concerted, sequential and more or less predominant action of various protective resources: by mechanical filtration by the lymph glands; by the formation of various specific antibodies, through phagocytosis, and so on. In the end-result it is always the nature as well as the vigor and completeness of the patient's reaction to the infection which determines the result.

It is fallacious, as Churchman comments, to assume that in bacteriemia the bacteria travel round and round in the blood stream reproducing as they go, and that injected antibacterial substances travel with them. It is more correct, and more likely, that there is an ebb and flow, a rise and fall in the bacterial content of the blood in accordance with repeated influxes from the primary focus, as well as from secondarily established multiple foci, and the repeated attempts at cleansing of the blood stream by the defensive agencies.

Admitting that mercurochrome and other comparable preparations can be introduced into the circulation only in relatively small amounts and, in terms of the blood volume, in very low concentrations (1:10,000 or over); that they do not remain long in the blood as such, that they are soon removed; and that they can hardly remain in contact with the bacteria long enough to exert a continuous or even prolonged bactericidal effect in terms of the infection as a whole, it seems quite probable that their bactericidal properties may have been given undue importance in past considerations.

Practically all bacteria, moreover, upon destruction and disintegration liberate greater or lesser amounts of endotoxin. Consideration of the inherent possibilities of the sudden liberation of massive doses of endotoxin consequent upon a rapid and complete sterilization of a heavily infected blood stream warrants the assumption that this may not always be desirable.

That such sudden absorption of endotoxin occurs can confidently be assumed from the character of the reaction occurring when the bactericidal action of mercurochrome is marked, reactions characterized by chills, fever, sweating and, in some cases, the picture of shock.

In the case reported all these factors were taken into consideration; the *bacteriostatic* rather than the bactericidal action of mercurochrome was sought; and an endeavor was made to determine the frequency and amount given by a study of the blood count as eliciting information not readily apparent from the clinical picture.

The report of the case is made possible by the courtesy of Drs. Theodore Sensemen and James H. Mason.

CASE 1.—C. G., aged forty, truck driver, on the night of March 13, 1926, while working at the rear of his truck, was struck by an automobile. He was admitted to the hospital suffering from shock and complaining of pain and loss of function in the left leg and thigh. Examination revealed a compound, comminuted fracture of the middle third of the left femur, a small puncture wound on the posterior aspect of the left thigh, and multiple abrasions over the site of the fracture.

The x-ray report of March 14 reads: "Oblique fracture of the left femur just above the condyle. Step fracture about three inches above the knee."

A second picture on March 15 showed a dishing of the femur, the middle fragment being above the upper and lower fragments, with marked comminution of all three.

The fracture was again reduced and the leg placed in a double inclined plane, x-ray showing the reduction to be satisfactory. Some difficulty was experienced, however, in keeping the fragments in good position and on March 25 an open reduction was done and a Lane plate inserted. Two days later, without chill or rigor, there was a sudden rise of temperature to 101.5° followed by a fall the next day to 99°. At this time the patient complained of much pain in the leg which did not cease.

On April 10, fifteen days after the operation, there was a considerable amount of drainage from the operative wound. The drainage continued and the temperature gradually rose, the patient complaining unceasingly of pain and gradually becoming drowsy.

On April 16, three weeks after operation, the temperature suddenly rose to 103° accompanied by a chill. At this time there was a large amount of seropurulent drainage. The temperature during the next few days ranged from 99° to 102° and the patient was restless, irritable, and without appetite. On April 21, five weeks after the injury, three weeks after operation, and five days after what appears to have been the initial invasion (April 16) the temperature rose to 105°. The pain in the leg was not now so marked, the patient possibly being too toxic to complain as bitterly, and the drainage was greatly reduced in amount.

On April 22nd, the temperature was 105°-106° and the patient delirious. The leucocyte count was 16,400, with 81 per cent of neutrophiles and a leucocytic index of 6.8. A blood culture on this date, within twenty-four hours gave a marked turbidity in 200 c.c. of bouillon, the growth being a pure culture of nonhemolytic streptococci.

I first saw the patient on April 23. The temperature ranged from 103° to 104°; the patient was delirious, pallid, covered with a clammy sweat, and in very poor condition. A blood culture showed in twenty-four hours eleven colonies per c.c. of nonhemolytic streptococci.

The patient was, obviously, in a very desperate state and the prognosis grave. The use of mercurochrome-220 soluble was decided upon but with a definite departure from the strength and frequency of dosage usually advocated, based upon the belief that the attempt to sterilize the blood stream by chemotherapy constituted only a part of the story and that sudden, massive sterilization was not ipso facto necessarily desirable and in this case definitely contraindicated.

In view of the massive infection present in this patient, as shown by the heavy growth in the blood culture, and in view of his desperate condition, it seemed obvious that any at-

tempt at massive production of bacterial death and disintegration must lead inevitably to overwhelming the patient by the sudden liberation and absorption of a large dose of endotoxin.

The object in view was to produce, if possible, a definite degree of *bacteriostasis* rather than bacteriolysis, and to produce the minimum of reaction. Accordingly 5 c.c. of 1 per cent mercurochrome was injected intravenously just after taking a blood culture.

Three hours later, at 5 P.M., the patient had a severe chill and the temperature rose to 103°. At 4 A.M., April 24, the temperature had fallen abruptly to 97°, the patient was sweating profusely, the pulse weak, and the general condition that of collapse, this sequence of events being undoubtedly due to the liberation and absorption of streptococcal endotoxin.

On entering the ward on the morning of April 24 the change in the general appearance of the patient was startling and dramatic; his mind was clear and he was engaged in reading the paper. His temperature at 9 A.M. was 99° but at 11 A.M. was rising. The blood culture taken the day before previous to the injection showed ten colonies per c.c. of nonhemolytic streptococci.

At 11:30 A.M. a blood culture was taken and 5 c.c. of 0.5 per cent mercurochrome injected through the same needle, this weaker solution being chosen because of the severity of the reaction after the first injection. By midnight the temperature had risen to 105° accompanied by transient spells of delirium and numerous chills and at 6 A.M. on April 25 had fallen to 100° accompanied by profuse sweating.

A leucocyte count April 25 showed 15,150 with 97 per cent neutrophiles and a leucocytic index of 32.5; on the 26th the white count was 11,250, neutrophiles 84 per cent, and leucocytic index 5.

The drainage from the wound, meantime, was small in amount, seropurulent, and showed on culture nonhemolytic streptococci, Staphylococcus aureus, and bacilli of Friedlander type. In an endeavor to attack the focus from which the blood stream infection was occurring and recurring, the wound was frequently freely irrigated with the mercurochrome-acetone solution described by Scott and Hill[7] diluted half strength.

On April 27 the white count was 11,276 with 76 per cent neutrophiles and a leucocytic index of 3.6. The culture taken before the injection April 25 showed no growth after four days' incubation.

The temperature range was from 99° to 101° and the patient was apparently successfully battling his infection.

On the 28th, while his mental condition was somewhat hazy, the general condition seemed good, the temperature at 8 A.M. being 99° leucocytes 12,100, neutrophiles 73 per cent and leucocytic index 3.

At 2 P.M., however, the temperature suddenly rose to 103°. An immediate blood count showed a leucocytosis of 12,650 but a neutrophilic increase to 86 per cent, the leucocytic index being 6.6.

An intravenous injection of 3.5 c.c. of 0.5 per cent mercurochrome was given at 2:20 P.M., a blood culture being first taken. Within a few hours the temperature rose to 104°, dropping to 97.5° at 5 A.M., and rising to 100° by 8 A.M. the next morning.

The blood culture of the 28th gave 2 colonies per c.c. of streptococci. The white count on the 29th was 12,250, neutrophiles 69 per cent, leucocytic index 2.6. A blood culture on the 30th was sterile.

The general condition remained satisfactory for some time; there was free drainage from the wound and a Carrel-Dakin drip was inserted and on May 5 a culture from the wound showed only a few staphylococci.

The general progress of the case was now very satisfactory in every way and an uninterrupted convalescence was expected. On May 13, however, the patient had a chill at 5:30 A.M. and at 2 P.M. the temperature had risen to 103⅖° and 3.5 c.c. of 0.5 per cent mercurochrome was injected intravenously after first taking a blood culture. There was no perceptible growth in this culture until after four days' incubation when streptococci were recovered in small numbers.

At 11 P.M., eight hours after the injection, the temperature had fallen to 98° and the following day the blood count showed 10,450 white cells and 87 per cent neutrophiles with a leucocytic index of 7.

On May 15 the count was 9,300 with 74 per cent neutrophiles and a leucocytic index of 2.9.

The drainage from the wound was very slight by this time and the Dakin solution was replaced by a saline drip.

An x-ray at this time showed extensive erosion of the femur in the neighborhod of the plate the upper screw of which appeared to have pulled out. The lower portion of the upper fragment of the lower fracture showed an area of absorption suggesting the presence of a pocket of infection.

On June 8, the condition of the wound and patient being good, the plate was removed under gas anesthesia. No pockets of infection were found.

The wound healed nicely, no further events took place to complicate convalescence, and the patient was discharged on June 26. He is now at work with very good functional results.

It is to be noted that the total volume of mercurochrome injected in this case was 17 c.c., the total mercurochrome content being 0.11 gm., all quantities being much below the amounts usually given in a single dose. The frequency of dosage, moreover, was not arbitrary but determined by the leucocytic as well as the clinical picture as indicating the progress of the battle between the patient and his bacterial antagonists.

It is obvious that the result was not entirely dependent upon nor even markedly influenced by the bactericidal activity of the mercurochrome and that its bacteriostatic effect was an important if not the main factor.

There is little doubt that, in view of the general and desperate condition of the patient when treatment was begun, he would not have withstood the reaction consequent upon the administration of large or frequently repeated injections of mercurochrome, without taking into consideration the possibility of producing mercurial poisoning.

The fact that the focus of infection from which invasion of the blood stream occurred was accessible and amenable to treatment was also of importance in the favorable outcome.

The following conclusions are suggested from this experience:

1. The treatment of bacteriemia by the intravenous injection of mercurochrome-220 soluble should not be directed solely toward the immediate production of a therapia sterilans magna.

2. The bacteriostatic effect of the drug is of equal importance with its bactericidal activity.

3. From these premises it follows that the strength and amount of solution injected are not to be arbitrarily selected on the basis of the degree of bactericidal effect it may be possible to produce but should be determined by the clinical and bacteriologic picture of the particular case.

4. The frequency of dosage should be determined by the leucocytic index as indicating the progress of the battle between the patient and the invading bacteria.

5. Massive and frequent intravenous doses of mercurochrome-220 soluble are not necessary as a routine method of treatment.

6. The sudden absorption of massive doses of endotoxin as a result of sudden and massive bacterial destruction in the blood stream possesses elements of danger and should be avoided.

7. The production of some degree of reaction is to be sought for because of the therapeutic value of protein shock.

8. It is possible to sterilize the blood stream in the presence of streptococcemia by the injection of small amounts of mercurochrome at irregular intervals, the sterilization probably being due to the opportunity afforded by the induced bacteriostasis for the mobilization of the resisting powers of the patient rather than to the bactericidal effect of the drug.

9. Treatment of an accessible focus of infection is an important element and should not be neglected.

10. In view of the above premises both the strength of the dose and the frequency of administration of mercurochrome solutions should be modified.

REFERENCES

[1]Walker, J. E.: Effect of Mercurochrome-220 Soluble On The Germicidal Properties of Fresh Defibrinated Blood, Arch. Path. and Lab. Med., February, 1926, i, 199-203.

[2]Churchman, J. W.: Intravenous Use of Dyes, Jour. Am. Med. Assn., Dec. 12, 1925, lxxv, 1849.

[3]Young, J. W., Scott, W. W., and Hill, J. H.: Use of Mercurochrome By Mouth as a Urinary and Intestinal Antiseptic, Jour. Urol., September, 1924, iii, 237.

[4]Hill, J. H., and Bidgood, C. Y.: Effect of Intravenous Injection of Mercurochrome on the Kidneys, Bull. Johns Hopkins Hosp., December, 1924, xxv, 409.

[5]St. George, A. V.: Treatment of Sepsis With Mercurochrome-220 Soluble Report of Necropsies In Five Cases, Jour. Am. Med. Assn., Dec. 26, 1925, lxxv, 2005.

[6]Corper, J. H.: Pathologic Changes in the Lung from the Use of Mercurochrome-220 Soluble, Jour. Am. Med. Assn., July 25, 1925, lxxxv, 256.

[7]Scott, W. W., and Hill, J. H.: Presentation of a Pre-Operative Skin Disinfectant, an Alcohol-Acetone-Aqueous Solution of Mercurochrome, Jour. Urol., August, 1924, xiv, 135.

LABORATORY METHODS

THE DETERMINATION OF SUGAR IN NORMAL URINE*

By Mark R. Everett, Ph.D., and Mrs. M. O. Hart, B.Sc., Norman, Okla.

METABOLIC investigations have figured largely in the recent revival of the question of the nature of sugar in normal urine. Our original purpose was to investigate the nature of this material by isolation experiments. However, literature concerning the comparative reliability of the most commonly employed analytic methods was so meager that it seemed best to first study comparative analytic values and their bearing upon existing knowledge of the nature of these substances.

Limiting ourselves to methods which had already merited some attention and which could be used advantageously for a long series of determinations, we chose the methods of Benedict and Osterberg,[1] Folin and Berglund,[2] and Sumner.[3] In each case the latest published modification was employed. We call attention to the fact that the Sumner method used by us is essentially different from that used by Greenwald, Samet and Gross.[4]

Greenwald, Samet and Gross[4] compared the glucose equivalents of several pure sugars by the analytic methods under consideration, but this is only one phase of the analytic merit of the same methods when applied to urinary analysis. Sumner[5] compared one of his earlier methods with that of Benedict and Osterberg.[1] Together with the analysis of urine from one person by Greenwald, Gross and Samet,[6] this is the only comparative data available for the methods investigated by us.

A review with bibliography on the nature of sugar in normal urine is given by Greenwald, Gross and Samet.[6] Since that time Höst[7] and Lund and Wolf[8] have presented additional data to show that this sugar is not glucose.

According to recent investigations[6, 9, 10] sugar of normal urine must be regarded as a variable mixture of substances. The accurate determination of such mixtures is a difficult problem and there is danger in attempting to draw quantitative conclusions from analysis of a limited number of urines. Our data represents several hundred analyses on urine from fourteen healthy men and women, twenty to forty-three years of age. We feel, however, that due to the variable and unknown nature of these reducing substances, we are not justified in claiming more than a qualitative significance for our results. The same criticism applies to other published data. We have calculated our results only to the nearest per cent, believing that greater accuracy is impossible.

*Read before the Fifth Annual Convention of the American Society of Clinical Pathologists at Dallas, Texas, April 15, 16 and 17, 1926.

From the Department of Biochemistry and Pharmacology, University of Oklahoma, Norman.

EXPERIMENTAL

Since our work was entirely of a comparative nature, the analyses were made under strictly comparable conditions. The sugar standards were made by dilution of the same stock solution of anhydrous glucose, C. P., and were preserved with saturated benzoic acid solution. After proper dilution these standards were compared by the Folin-Wu method and were found to be exactly equivalent.

In the preparation of Sumner's reagent 3, 5-dinitrosalicylic acid, C. P., Eastman Kodak Co., was used. Boneblack was prepared from commercial boneblack according to the directions of Benedict and Osterberg.

Unless otherwise stated, the analyses were made as soon as possible on one and one-half to four-hour period urines, voided by persons who during the previous twenty-four hours had eaten no toasted food or other food now known to give rise to large amounts of "foreign carbohydrates" in the urine. In this way we hoped to obtain samples of urine having a normal composition. Whenever precipitates appeared (as in alkaline urines) they were carefully suspended before taking the analytic sample.

Checks obtained upon the data reported never varied more than 1 per cent, with an average of 0.5 per cent, for the Folin-Berglund and the Sumner methods and never more than 2.2 per cent, with an average of 1.0 per cent, for the Benedict-Osterberg method. In the latter method the color produced

TABLE I

COMPARATIVE VALUES ON NORMAL URINE

NO.	METHOD 1 MG./C.C.	METHOD 2 MG./C.C.	METH. 1/METH. 2 PER CENT		REMARKS
	Folin-Berglund	Benedict-Osterberg			
24	0.523	0.572		109	18 hr. Urine
25	0.617	0.673		109	" " "
1	0.559	0.815		146	
9	0.696	1.183		170	5½ hr. Urine P_H 7.4
11	0.537	0.800		149	
			Average	137	
			Average of 1, 9, 11	155	
	Folin-Berglund	Sumner			
27	0.729	0.766		105	
28	0.531	0.552		104	
29	0.328	0.350		107	
1	0.559	0.600		107	
9	0.696	0.743		107	5½ hr. Urine P_H 7.4
11	0.537	0.556		104	
12	0.269	0.261		97	
13	0.635	0.592		93	
			Average	103	
			Average of 1, 9, 11	106	
	Sumner	Benedict-Osterberg			
30	0.952	1.645		173	
1	0.600	0.815		136	
2	0.502	0.746		149	
9	0.743	1.183		159	5½ hr. Urine P_H 7.4
11	0.556	0.800		144	
			Average	152	
			Average of 1, 9, 11	146	

by the same amount of reducing substance is less intense and harder to compare than in the first two methods. This fact may explain the greater divergence in duplicate analyses by the Benedict-Osterberg method.

Analyses by the Benedict-Osterberg and Sumner methods were made on filtrates from urine treated with boneblack. The urine samples were acid in all but one case (No. 9).

The Folin-Berglund method and the Sumner method, employing boneblack, gave quite similar results, variations of 7 per cent or less being found (Table I). In the eight direct comparisons of these two methods on different urines an average of 3 per cent higher values for the Sumner method was obtained.

Difference in the analytic values might be caused by an adsorption of sugar by Lloyd's reagent or by boneblack. Sumner[5] found lower values for urine treated with boneblack and attributed this effect partly to a loss of sugar by adsorption. He found the adsorption of glucose more marked in neutral than in acid solution. If Lloyd's reagent adsorbs no sugar and if loss of sugar is the only factor concerned, we would expect consistently lower values by the Sumner method. Such errors, if they do exist, may be counterbalanced by increased color production from other sources.

We tried the effect of thirty noncarbohydrate constituents of normal urine upon the reagents used in these methods. The presence of aldehydes, dioxybenzenes, cystine, or ammonium salts, large amounts of each of which gave colors similar to the color given by glucose, appeared to be the most likely source of error for the Sumner method. However, none of these substances gave colors when present in amounts likely to be found in normal urine. Ammonium salts in concentrations corresponding to those found in certain pathologic samples of urine gave an appreciable color. Of the substances mentioned, aldehydes were the only ones yielding an appreciable color with the Folin-Wu reagents. On the other hand, uric acid, allantoin, creatine, and creatinine also give colors with the Folin-Wu reagents, but these substances are removed by Lloyd's reagent. Moreover Folin and Wu[11] state that their older reagent is unaffected by 0.05 per cent of uric acid, creatine or creatinine in the analytic sample. They do not say whether this is true for the new reagent or whether the somewhat larger amounts of creatinine and uric acid, at times encountered in urinalysis, are equally harmless. We found that creatinine and uric acid in the concentrations occasionally found in very concentrated urine were not sufficietly removed by Lloyd's reagent to prevent the appearance of a slight color. However, these are not valid objections to the Folin-Berglund procedure as such urine can and should be diluted before the analysis is made. Concentrated mammalian urine requires the same treatment because of the larger amounts of allantoin present. The small amount of allantoin in human urine has no effect on the Folin-Wu reagents. Sumner says his reagent is "almost completely specific for reducing sugars" and we agree that it is more specific than the Folin-Wu reagents, as far as interference by creatine, creatinine, uric acid, or allantoin is concerned. Our present knowledge permits the conclusion that both methods are sufficiently specific to give identical values.

A more probable cause of the different values might be an increased color production by substances which do not themselves give colors but which increase the color given by sugars. Sumner discovered such an effect for phenols with his earlier reagent. The same relations may apply to all sugar methods for other urinary constituents.

Finally the individual sugars of normal urine may have different glucose equivalents for the two methods. Greenwald, Samet and Gross[4] have determined the glucose equivalents of several pure sugars for the two methods but they used the older Sumner reagent.[5]

In comparison with these two methods the Benedict-Osterberg method gave higher and more variable values. Analyses by Greenwald, Gross and Samet[6] of urine from a single individual reveal the same tendency. We calculated the ratio of Benedict-Osterberg to Folin-Berglund values reported by them and found the following averages:

	BENEDICT-OSTERBERG / FOLIN-BERGLUND	VARIATIONS
Carbohydrate-rich diet	139.5 per cent	121 per cent to 155 per cent
Carbohydrate-poor diet	139 per cent	121 per cent to 156 per cent
Fat-poor diet	169 per cent	137 per cent to 220 per cent
Varied diet	148 per cent	116 per cent to 173 per cent
Glucose and fat, or protein diet	155 per cent	122 per cent to 212 per cent

We could find no definite relations between these ratios and the dietary variations. Urine voided after glucose meals gave sometimes greatly increased and sometimes greatly decreased ratios. No apparent significance could be found in the relative values given by the two Benedict-Osterberg methods. Benedict and Osterberg[1] claim excellent agreement for these methods, but one can find variations of 20 per cent in their own reported comparisons, and as much as 65 per cent in the analyses of Greenwald, Gross and Samet. These divergences are certainly greater than those found by us between the Folin-Berglund and Sumner methods.

The factors previously mentioned might cause the high Benedict-Osterberg values. We found that large amounts of aldehydes and dioxybenzenes gave an intense color in their procedure. However, when present in concentrations likely to be found in normal urine, these substances gave no color. The color of the alkaline picrate solution was also affected slightly by a variety of other substances. The presence of certain amino derivatives, ammonium salts, ethyl acetoacetate and sodium bicarbonate caused a fading of the color of the reagent itself. The fading effect was produced by a few of these substances when present in amounts likely to be found in normal urine. While one may attribute the comparatively greater variations of the Benedict-Osterberg values to these effects, it is necessary to search elsewhere for the cause of the higher values.

There is no doubt that all the sugars examined by Greenwald, Samet and Gross, except glucosamine, have higher glucose equivalents for the Benedict-Osterberg method than for the Folin-Berglund method. It is also probable that, just as in the case of the older Sumner method, increases of color are produced in the Benedict-Osterberg method by substances which them-

selves yield little or no color. Until the cause of the higher Benedict-Osterberg values is definitely determined, glucose equivalents of sugars are of doubtful value in elucidating the nature of sugar in normal urine.

Because of their great divergence from values secured by the methods of Folin and Berglund and of Sumner, we consider Benedict-Osterberg values less reliable and more difficult of interpretation in metabolic experiments. This divergence is an important matter in metabolic experiments where differences of sugar excretion are measured. Some of the conflicting results, in such investigations, may be explained on this basis (cf. Benedict and Osterberg[12]).

The Benedict-Osterberg method is the most difficult of the three methods being discussed and the Sumner method is the simplest and shortest. There is a greater proportional color range for the Sumner method than for the Folin-Berglund method.

TABLE II

COMPARATIVE EFFECT OF NORITE AND BONEBLACK

NO.	METHOD	BONEBLACK MG./C.C.	NORITE MG./C.C.	PER CENT	P_H	REMARKS
	(a) Acid Urine					
4	Sumner	0.446	0.415	93	4	
8	"	0.602	0.704	117	4.5	
5	Benedict-Osterberg	0.585	0.588	101	4	
7	" "	1.099	1.031	94	4	6 hr. Urine
	(b) Alkaline Urine					
4	Sumner	0.508	0.568	112	9.5	
8	"	0.735	0.806	110	8.5	
5	Benedict-Osterberg	0.549	0.559	102	9.5	
7	" "	0.913	0.976	107	9	6 hr. Urine

In the experiments recorded in Table II, the urine was made acid or alkaline in each case by the addition of not more than one drop of concentrated hydrochloric acid or of 55 per cent sodium hydroxide solution per 10 c.c. of urine, so that the dilution effect was always less than 0.5 per cent and the difference in the dilution of the same urine never more than 0.25 per cent. In acid urine the use of norite gave variable results for both Sumner and Benedict-Osterberg methods, while in alkaline urine it led to higher values for both methods, especially for Sumner's method. The filtrates from alkaline urine were decidedly more yellow than other norite or boneblack filtrates. The P_H of the samples was purposely beyond that encountered in normal urine in order to get a magnified effect. In all other analyses we adhered to the use of boneblack as the more desirable procedure.

CHANGES IN URINE ON STANDING

It soon became apparent that occasional changes in sugar values occurred in urine which had been standing several hours.

As was to be expected the greatest changes were encountered with long period samples. Retention in the bladder and standing of part of the samples in flasks gave increased opportunities for chemical changes and bacterial action. What part bacterial action might play in these changes could best be determined by the study of changes in preserved urine. It was first

TABLE III

NO.	TIME AFTER VOIDING	FOLIN-BERGLUND MG./C.C.	CHANGE PER CENT	BENEDICT-OSTERBERG MG./C.C.	CHANGE PER CENT	REMARKS
21	2 hours	0.439		0.648		18 hr. Urine
	6 "	0.506	+15	0.704	+9	
22	2 hours	0.480		0.596		16 hr. Urine
	6 "	0.404	−16	0.721	+21	
23	2 hours	0.295		0.393		12 hr. Urine
	6 "	0.291	−1	0.342	−13	
				Sumner		
27	2 hours	0.729		0.766		
	4.5 "	0.721	−1	0.760	−1	
29	2 hours	0.328		0.350		
	4.5 "	0.327	0	0.350	0	
		Benedict-Osterberg				
30	2 hours	1.645		0.952		
	3 "	1.613	−2	0.948	0	

necessary to find out whether the preservatives themselves had any effect upon the analytic values.

Portions of the original urine were shaken vigorously with the preservatives. As soon as the chloroform and toluene had separated, analyses were made simultaneously on all samples. Toluene had the least effect upon the values obtained by the Folin-Berglund method (3 per cent lower values); thymol had slightly more effect (5 per cent lower values); while greater variations were encountered with chloroform. The lower values for toluene-preserved urine were not due to the inclusion of this preservative in the analytic sample as shown by the Sumner values for the same samples. Values secured by the Sumner method appeared to be least affected, being practically identical for the original urine and all preserved samples. The Benedict-Osterberg values were also very little affected except in the case of chloroform-preserved samples which showed variations similar to those encountered with the Folin-Berglund method. Toluene and thymol, rather than chloroform, are the preservatives to be recommended for this purpose.

From the experiments recorded in Table V it is evident that changes are not entirely prevented by preservatives. The first two series of analyses are especially interesting. Urine No. 12 showed decreased values for unpreserved samples by both Folin-Berglund and the Sumner methods. Values for toluene-preserved samples showed approximately the same decrease with the Folin-Berglund method, but slightly increased values with the Sumner method. Urine No. 13 showed no appreciable changes when unpreserved but showed increased values when preserved with toluene. There is no doubt that bacteria were present in both unpreserved samples. Bacterial growth was prevented in the toluene-preserved urines for forty-eight hours. There is some doubt as to the ability of toluene to preserve urine indefinitely.

It seems evident from our data that bacterial action can remove part of the "sugar" and that a simultaneous liberation of increased amounts of reducing substances may at times counteract these effects. Since changes occur in toluene-preserved urine it is not possible to postpone the analysis without securing inaccurate results. The best plan in metabolic experiments is to analyze samples immediately and to deduce twenty-four-hour values from analysis of a number of short period samples.

TABLE IV

EFFECT OF PRESERVATIVES

NO.	METHOD	ORIGINAL MG./C.C.	CHLOROFORM MG./C.C	PER CENT	THYMOL MG./C.C.	PER CENT	TOLUENE MG./C.C.	PER CENT	P_H	REMARKS
12	Folin-Berglund	0.269					0.273	101	6.9	
13	"	0.635					0.635	100	6.5	
1	"	0.559					0.542	97	6.7	
2	"	0.571	0.661	116	0.537	94	0.523	92	6.5	
9	"	0.696	0.625	90	0.672	97	0.645	93	7.4	5½ hr. Urine
11	"	0.537	0.650	121	0.510	95	0.533	99	6.8	
			Average		Average	95	Average	97		
14	Benedict-Osterberg	0.746	0.775	104	0.769	103	0.749	100	6.5	
1	"	0.815					0.803	99	6.7	
9	"	1.183	1.149	97	1.143	97	1.143	97	7.4	5½ hr. Urine
11	"	0.800	0.870	109	0.833	104			6.8	
			Average	103	Average	101	Average	99		
12	Sumner	0.261					0.258	99	6.9	
13	"	0.592					0.595	101	6.5	
14	"	0.502	0.499	99	0.511	102	0.510	102	6.5	
1	"	0.600					0.602	100	6.7	
9	"	0.743	0.741	100	0.741	100	0.738	99	7.4	5½ hr. Urine
11	"	0.556	0.559	101	0.556	100	0.556	100	6.8	
			Average	100	Average	101	Average	100		

TABLE V

CHANGES IN PRESERVED URINE

TIME AFTER VOIDING	FOLIN-BERGLUND				SUMNER			
	ORIGINAL MG./C.C.	CHANGE %	TOLUENE MG./C.C.	CHANGE %	ORIGINAL MG./C.C.	CHANGE %	TOLUENE MG./C.C.	CHANGE %
			Urine No. 12, P_H 6.9					
½ hour	0.269		0.273		0.261		0.258	
3½ hours	0.262	−3	0.262	−4	0.258	−1	0.256	−1
25½ hours	0.253	−6	0.250	−8	0.237	−9	0.265	+3
	Unpreserved sample cloudy							
			Urine No. 13, P_H 6.5					
1 hour	0.635		0.635		0.592		0.595	
5⅔ hours	0.641	+1	0.647	+2	0.599	+1	0.610	+3
25½ hours	0.635	0	0.672	+4	0.588	−1	0.629	+6
	Unpreserved sample cloudy							

TIME AFTER VOIDING	FOLIN-BERGLUND		BENEDICT-OSTERBERG		SUMNER	
	TOLUENE MG./C.C.	CHANGE %	TOLUENE MG./C.C.	CHANGE %	TOLUENE MG./C.C.	CHANGE %
			Urine No. 1, P_H 6.7			
1 hour	0.542		0.803		0.602	
24 hours	0.533	−2				
27 hours			0.995	+24	0.615	+2

COMPARISON OF VALUES ON ACID AND ALKALINE URINE

Our last experiments were directed toward answering the question: Is the hydrogen-ion concentration of urine partly responsible for changes in the sugar content of urine upon standing? The urine was made acid or alkaline in the manner described previously. The P_H of the samples was purposely altered beyond the limits to be found in normal urine (especially in the case of alkaline urine) in order to magnify the results. The effect of P_H on the analytic method employed is given in Table VI.

TABLE VI

NO.	METHOD	ORIGINAL MG./C.C.	ACID MG./C.C.	ALKALINE MG./C.C.	ALKALINE / ACID P_H PER CENT		REMARKS
3	Folin-Berglund		0.792	0.808	102	4 and 9	
10	"		0.365	0.432	118	4 and 9	
11	"	0.537	0.506	0.540	107	4 and 9.5	
				Average	109		
5	Benedict-Osterberg		0.585	0.549	94	4 and 9.5	
7	"		1.099	0.913	83	4 and 9	6 hr. urine
				Average	88½		
4	Sumner		0.446	0.508	114	4 and 9.5	
8	"		0.602	0.735	122	4.5 and 8.5	
11	"	0.556	0.526	0.562	107	4 and 9.5	
				Average	114		

All analyses on urine No. 11 were made at the same time. The filtrates in the Folin-Berglund procedure were always acid, P_H below 4 for the acid urines and about 6 to 6.5 for the alkaline urines.

The Folin-Berglund and Sumner methods gave higher values for alkaline urine than for the acid urine but, in the case of urine No. 11, at least, the cause of this phenomenon was a lowering of the values for acid urine. The values for alkaline urine were practically identical with those secured

on the original urine which was slightly acid, (P_H 6.8). We may say, therefore, that an acid reaction has a tendency to give lower values with these two methods. The diminutions in acid urine are exactly the reverse of the expected results, if the sugar is adsorbed by boneblack in the same fashion as glucose (cf. Sumner[3]). With the Benedict-Osterberg method, we noticed exactly the opposite tendency, namely, lower values for alkaline urine. These results might be produced by differences in the adsorption of reducing substances by boneblack and Lloyd's reagent, or by variations in the reduction process itself. Of course exaggerated values were obtained by these experiments but it is very probable that similar, though smaller, deviations may be expected in normal urines where the P_H varies considerably.

The changes in sugar values produced by heating acid and alkaline urine are helpful in differentiating between the effect of the hydrogen-ion concentration on the analytic procedure and on the "sugar" of urine itself (Table VII).

TABLE VII

EFFECTS PRODUCED BY HEATING FOR 10 MINUTES AT 100° C.

NO.	METHOD	BEFORE HEATING MG./C.C.	AFTER HEATING M.G./C.C.	CHANGE %	P_H	TIME AFTER VOIDING	REMARKS
			ACID URINE				
3	Folin-Berglund	0.792	0.833	+ 5	4	2 hours	
10	" "	0.365	0.406	+ 11	4	1¼ hours	
11	"	0.506	0.563	+ 11	4	3 hours	
			Average	+ 9			
5	Benedict-Osterberg	0.585	0.610	+ 4	4	¾ hour	
7	"	1.099	1.105	+ 0.5	4	2½ hours	6 hr. Urine
			Average	+ 2			
4	Sumner	0.446	0.467	+ 5	4	2 hours	
8	"	0.602	0.606	+ 1	4.5	1½ hours	
11	"	0.526	0.529	+ 1	4	3 hours	
			Average	+ 2			
			ALKALINE URINE				
3	Folin-Berglund	0.808	0.816	+ 1	9	2 hours	
10	" "	0.432	0.430	0	9	1¼ hours	
11	" "	0.540	0.392	− 27	9.5	3 hours	
			Average	− 9			
5	Benedict-Osterberg	0.549	0.365	− 34	9.5	¾ hours	
7	"	0.913	0.840	− 8	9	2½ hours	6 hr. Urine
11	"	0.877	0.495	− 44	9.5	3 hours	
			Average	− 29			
4	Sumner	0.508	0.424	− 17	9.5	2 hours	
8	"	0.735	0.714	− 3	8.5	1½ hours	
11	"	0.562	0.424	− 25	9.5	3 hours	
			Average	− 15			

The urine was heated in volumetric flasks, cooled and made up to the original volume. All methods gave increased values with heated acid urines. The Folin-Berglund method was especially sensitive to this change which was exactly opposite to the effect produced by the mere presence of acid on the values given by the Folin-Berglund and Sumner methods. There was a well-marked tendency to decreased values with heated alkaline urine, especially with the Benedict-Osterberg method.

TABLE VIII

	TIME AFTER VOIDING	ACID URINE MG./C.C.	CHANGE %	ALKALINE URINE MG./C.C.	CHANGE %
		Folin-Berglund Method			
Urine No. 3, P_H 4 and 9	2 hrs.	0.792		0.808	
	45 hrs.	0.792	0	0.808	0
	123 hrs.	0.800	+ 1	0.792	- 2
Urine No. 10, P_H 4 and 9	1.25 hrs.	0.365		0.432	
	49.25 hrs.	0.468	+28	0.441	+ 2
		Benedict-Osterberg Method			
Urine No. 5, P_H 4 and 9.5	1 hr.	0.585		0.549	
	93 hrs.	0.749	+28	0.502	- 9
Urine No. 7, P_H 4 and 9, 6 hr. urine	2.5 hrs.	1.099		0.913	
	56 hrs.	1.130	+ 3	0.847	- 7
	128.75 hrs.	0.741	-33	0.633	-31
		Sumner Method			
Urine No. 8, P_H 4.5 and 8.5	1.5 hrs.	0.602		0.735	
	25.75 hrs.	0.588	- 2	0.699	- 5

The object of the experiments recorded in Table VIII was to determine whether similar changes occurred in these samples upon standing. The samples were preserved with toluene.

The expected increases in acid urine were sometimes obtained. In one sample analyzed by the Benedict-Osterberg method there was later a marked decrease in sugar values. However, in samples which have stood as long as this one, the preservative value of toluene against microorganisms may be questioned. As a rule, the alkaline samples showed the expected decreased values. We are convinced that changes dependent upon P_H occur in preserved samples of urine and that the best procedure is to analyze the urine at once (at least within three to four hours after the sample is voided). Samples which have to be kept longer must be preserved and kept at a low temperature to avoid changes in apparent sugar content.

The variations encountered recall the fact that sugar of normal urine is a variable mixture of substances and no two urines may be expected to behave exactly alike. Increased values in acid solutions after standing may be attributed to hydrolysis of "combined sugars" (perhaps disaccharides). Decreased values in alkaline solutions may be caused by the destruction of sugar, or of some substance which increases the color produced by sugar. Similarly, the occasional decreased values in acid solutions may be attributed to the destruction of some noncarbohydrate substance.

The present analytic methods determine the reducing power of urine in terms of equivalent glucose reduction under specific conditions. Any substances which are not adsorbed by Lloyd's reagent from acid solution or by boneblack from acid or slightly alkaline solution, and which reduce the sugar reagents or increase the reduction of these reagents by sugar, are included in the term "sugar of normal urine."

CONCLUSIONS

The methods of Folin and Berglund and of Sumner give approximately the same values for sugar in normal urine, while the Benedict-Osterberg method gives higher and probably less reliable values. There are several

factors which may be responsible for the differences in analytic values. Glucose equivalents for the urinary sugar may not be the same for the several analytic methods. There may be present in urine, substances which do not give colors themselves, but which increase or decrease the amount of color given by the sugar.

Somewhat irregular changes in the sugar content of normal urine take place after the sample is voided. These changes are of sufficient magnitude to affect the interpretation of published metabolic data. Preservation of urine with toluene does not entirely prevent such changes. Not all antiseptics are suitable for preserving the analytic samples.

The hydrogen-ion concentration of the urine appears to affect both the analytic result and the nature of the changes which occur in voided urine.

REFERENCES

[1]Benedict and Osterberg: Jour. Biol. Chem., 1921, xlviii, 51.
[2]Folin and Berglund: Jour. Biol. Chem., 1922, li, 209.
[3]Sumner: Jour. Biol. Chem., 1925, lxv, 393.
[4]Greenwald, Samet and Gross: Jour. Biol. Chem., 1924, lxii, 397.
[5]Sumner: Jour. Biol. Chem., 1924, lxii, 287.
[6]Greenwald, Gross and Samet: Jour. Biol. Chem., 1924, lxii, 401.
[7]Höst: Acta Med. Scand. Sup., 1924, vii, 180.
[8]Lund and Wolf: Biochem. Jour., 1925, xix, 538.
[9]Folin and Berglund: Jour. Biol. Chem., 1922, li, 213.
[10]Höst: Jour. Metabol. Research, 1923, iv, 315.
[11]Folin and Wu: Jour. Biol. Chem., 1919, xxxviii, 106.
[12]Benedict and Osterberg: Jour. Biol. Chem., 1923, lv, 769.

DISCUSSION

Dr. Frederic E. Sondern.—It is very evident that this communication is the result of a very extensive study. Dr. Everett may be complimented on this paper.

Dr. Wm. Taylor Cummins.—This is a subject in which we have been much interested in the Southern Pacific General Hospital, San Francisco. We have carried out the Sumner and Benedict colorimetric technics for normal urine sugar coincidently with the qualitative Benedict technic and have found in some instances that the latter gives figures twice as large as the former. The study of normal urine sugar was prompted sometime ago by the idea that some laboratory workers and clinicians were overestimating urine sugar in the qualitative Benedict technic. With this technic we place the tubes containing 5 c.c. of urine and 8 drops of Benedict solution in a beaker of boiling water for exactly five minutes. I wish to compliment Dr. Everett on his work in which we are much interested.

Dr. Mark R. Everett (closing).—In reply to Dr. Cummins I was speaking of a qualitative test. I have some very definite replies for Dr. Exton. He was speaking of an entirely different and older method which gave higher values. A new method has been devised and it is this method which I have been discussing. The values reported were secured on preserved samples of urine after four to twenty-four hours standing. I cannot recommend formalin as a preservative in sugar analysis. In regard to the question of the nature of these sugars, I have no doubt that sugar of normal urine is not glucose, but rather a mixture of reducing substances which are sugars and not other reducing substances as intimated by Dr. Exton. I wish to emphasize that the disappearance from urine of these reducing sugars is appreciable within twenty-four hours. I am very grateful for Dr. Exton's criticism.

A NEW MASK FOR USE IN BASAL METABOLISM DETERMINATIONS*

By Allan Winter Rowe, Ph.D., Boston, Mass.

THE development of types of apparatus for the determination of the basal metabolic rate, which did not enclose the subject within a closed respiration chamber, has necessitated the elaboration of apparatus for connecting the subject with the gasometer. The joints thus made must be gas tight, as leakage is only less prejudicial to accuracy in the open than in the closed circuit method. The importance of the problem and the failure of complete success which has attended its solution is amply attested by the numerous devices described in the literature. Briefly stated, gas tight connection must be made through the mouth, the nose, or through both together. In event of but one orifice being utilized, the other must be hermetically sealed.

A standard method in rather general use is the rubber mouthpiece of Denayrouse[1] consisting of a rubber plate disposed between the lips and teeth, an outlet tube of ample dimensions passing between the former and two rubber offsets from the main plate which are engaged between the latter. Occasionally a case is encountered in which the ample dimensions of the mouth plate are inadequate. Loss of teeth rarely, if ever, proves a complication. The complement of this mouthpiece is a nose clip permitting exact closure of the nostrils. Several designs are available and all are reasonably effective. A certain percentage of cases encountered possess a nasal configuration which requires much pressure to effect a proper seal. This may be so strait as to be painful, in which event the absolute basality of the test is lost. On the whole, however, in my opinion, the mouthpiece and nose clip constitute the method of election because of their simplicity, ease of adjustment, cleanliness, sterility, and psychologic effect, this latter a factor that should never be ignored.

A second type of partial connection makes use of nosepieces. As originally designed by Tissot[2] these consist of glass tubes with a symmetrical circular enlargement, one of which is forced into each nostril. In the studies at the Carnegie Nutrition Laboratory,[3] a first modification of these tubes involved the transformation of the terminal spheres to flattened ovoid form. Subsequently, small tubes were used with pneumatic shields made of thin rubber (dental dam) which were inflated after the tubes had been inserted in the nostrils. Mouth closure was effected by firm pressing of the lips, reinforced in those whose hirsute adornment would permit, with strips of adhesive plaster. Even in their last and blandest form the nosepieces, if gas tight, are definitely painful. Their use in a laboratory where scientific studies are being made on normals is feasible, but for clinical application and use

*From the Evans Memorial, Boston, Mass.

with the sick, they cannot be recommended. The discomfort entailed by their use destroys the basal state and leads to results which may be most misleading. Benedict and Benedict[4] have called attention to the serious error that may be induced by minor body movements. In a study on the metabolism during pregnancy[5] in which repeated observations were made on the same subject over considerable intervals of time, the writer found that the slight movements associated with nervous tension and discomfort might introduce an error to the magnitude of over 20 per cent. This observation was unconsciously verified by Baer[6] in a study on the same condition.

The use of a mask early suggested itself, and a variety of what may be termed "half-masks" were utilized. Boothby and Sandiford[7] use a rubber mask of rough triangular shape similar to that used for mine rescue work. This covers only the nose and mouth and is held in place by an elaborate network of tapes. A pneumatic rim which can be inflated they discard, owing to change of tension in the peripheral pressure and possible leakage. Carpenter (l.c.) finds this mask unsatisfactory and substitutes one of sheet lead in the form of a cone, luted to the face with plasticene. Other commercial apparatus have made use of various modifications of the half-mask attached to certain forms of anesthetic apparatus. With the half-mask, the mutations of contour of the human face make exact fitting a matter of much difficulty. The rubber masks require what at times is a painful pressure to render the joints gas tight, while the lead mask is patently ill adapted for clinical use.

The utilization of the full gas mask of warfare, covering the entire face, was first suggested by Bailey.[8] Using a gas mask designed for use by the French Army, he adapts it to current purposes by adding pressure pads of five-inch rubber sponges over the areas (temporal zones) where leaks are likely to occur. The apparatus is readily adjusted, gives a satisfactory closure, and most important, is comfortable.

Personal experience with the several types of apparatus here described led the writer to adopt the standard rubber mouthpiece and nose clip as most nearly conforming to the requirements of an active clinical service. As before stated, however, a small percentage of cases find it impossible to adapt themselves to the method. This group includes such types as the deep breathers with explosive expiration, mentally retarded adolescents with fluttering breath, and the group where the anatomic configuration of the nose makes closure either impossible or disturbingly painful. To meet these contingencies, a full mask seemed the most suitable device, as I had duplicated Carpenter's experience with the rubber half-mask. The war gas mask obviously possessed many advantages. Designed so to fit the face as to prevent leakage from the outside, it remained only to modify it to arrest any complementary leakage from within. Inquiry developed the fact* that samples of the perfected type of mask designed by Major Waldemar Kops for use with the A. E. F. were available. This mask† was secured in three sizes, an important provision when the variation of size of head is considered. The mask fastens by a series

*I take pleasure in acknowledging my indebtedness to Bradley Dewey, Esq., late Colonel, C.W.S., U.S.A., for most courteous and helpful assistance.

†I take the greatest pleasure in acknowledging my basic indebtedness to Major Kops, through whose generous gift the numerous masks used in this study were made available.

of elastic bands originating at the salient points of the periphery and uniting in a pad at the back of the head. The conventional slip buckle allows the independent regulation of the tension in each band and permits consequent accurate fit of the mask (see Fig. 1). To secure closure against leakage from the inside, a thin-walled rubber tube, 1¼″ in diameter, was cemented along the inner proximal edge of the entire circumference. At the superior point a small rubber tube was introduced, terminating in the ordinary bicycle tire valve. Through this the tube could be inflated to any degree of tension by a small hand pump* (see Fig. 2).

Fig. 1.

Fig. 2.

METHOD OF USE

The mask has been used with the Benedict-Collins type of closed circuit instrument.[9] As it has two outlets, however, it is equally adaptable to other types of apparatus, including all open circuit methods.

To apply the mask, it is adjusted to the head with the tube in the deflated state. Each elastic band is adjusted until the mask fits snugly and simple testing shows there is no appreciable inward leakage on inspiration. The patient next resting in a recumbent position (the mask is adjusted in a supported sitting posture), an interval is allowed to elapse to eliminate the effect of the earlier muscular effort. This also serves the purpose of familiarizing the subject with the mask, a not unnecessary precaution. The mask being comfortably and snugly adjusted, the hand pump is next attached to the

*For the time consuming experimentation necessary to prepare and fit this inner tube, I am indebted to the co-operation of the Davol Rubber Co., Providence, R. I.

tube and the latter inflated to the requisite tension. The pliable character of the tube and thus its easy adaptation to inequalities in the contour of the bearing surfaces renders it effective without discomfort. Soapsuds or grease may be used if there be reason to suspect leakage. Usually this has been found to be unnecessary. When the patient is resting comfortably the superior tube is connected with the apparatus and clamped off lightly while the latter is filled. During this part of the operation the patient breathes easily through the lower tube which is open. With the apparatus adjusted, the clamp is removed, and the lower tube closed by the rapid insertion of a

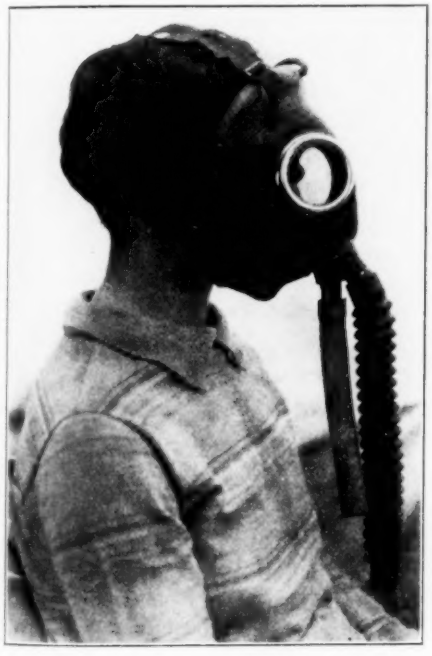


Fig. 3.

Fig. 4.

long tapered rubber stopper, well greased, and forced into the orifice to complete closure. Several minutes should now elapse—not less than four—to establish the gas equilibrium throughout the system. As the patient is in no discomfort, and as additional oxygen may be introduced at will, the element of haste is eliminated. At the expiration of the test, disconnection is made, and after deflation and loosening of the straps, the mask is readily removed. In the writer's experience, a heavy beard is the only inhibiting facial condition. Tests have been made successfully with subjects wearing small chin beards, as the bearing surface of the mask falls well toward the angle of the jaw (Figs. 3 and 4).

The mask possesses certain advantages for purposes other than that for

which it was originally designed. As it causes no discomfort it can be worn for long periods of time without introducing the fatigue and nervous tension which seemingly cannot be dissociated from the other forms. By the exercise of certain obvious precautions it can be used in other than a recumbent position and is adapted to tests of metabolism in other than the basal state.

In conclusion, I wish to say that in routine clinical work, for reasons given above, I regard the standard mouthpiece and nose clip as the apparatus of election. The present device offers an efficient and satisfactory means of securing measurements on those patients to whom the routine procedure is ill adapted. Further, it can be applied to measurements made under a variety of physical conditions.

SUMMARY

A gas mask modified for use in basal and other metabolism determinations is described.

REFERENCES

[1]Regnard: Recherches experimentales sur les variations pathologiques des combustions respiratores, Paris, 1879, p. 286.
[2]Tissot: Jour. de physiol. et de path. gen., 1904, vi, 688.
[3]Carpenter: A Comparison of Methods for Determining the Respiratory Exchange in Man, Carn. Inst. Pub., 1915, No. 216.
[4]Benedict and Benedict: Proc. Nat. Acad. Sci., 1924, 498. Bull. Soc. Sci. Hyg. Alim. xii, 1924, Nos. 8 and 9.
[5]Rowe, Alcott, and Mortimore: Am. Jour. Physiol., 1925, lxxi, 667.
[6]Baer: Am. Jour. Obst. and Gynec., 1921, ii, 249.
[7]Boothby and Sandiford: Laboratory Manual of the Technic of Basal Metabolic Rate Determinations, Saunders, Philadelphia, 1920.
[8]Bailey: JOUR. LAB. AND CLIN. MED., 1921, vi, 657; Jour. Biol. Chem., 1921, xlvii, 277.
[9]Benedict and Collins: Boston Med. and Surg. Jour., 1918, clxxviii, 567.

A NOTE ON THE METHOD OF DILUTING ANTIGENS FOR USE IN THE COMPLEMENT-FIXATION TEST FOR SYPHILIS*

BY ANNA C. MOORE, A.B., ALBANY, N. Y.

IN THE laboratory of the New York State Department of Health, two antigens are used routinely in the complement-fixation test for syphilis,—one, an acetone-insoluble antigen prepared by Bordet's[1] method and the other, a cholesterinized extract prepared by a method similar to that of Neymann and Gager.[2] The acetone-insoluble antigen is diluted by Bordet's method as follows: One part of antigen is evaporated to dryness. The dried residuum is then suspended in two and one-half parts of distilled water, the first cubic centimeter being added slowly with a 0.2 c.c. pipette and mixed as thoroughly as possible after the addition of each 0.2 c.c. The remainder of the water may be added rapidly, but the suspension must be thoroughly mixed. The appropriate amount of this suspension is then added to the amount of 0.85

*From the Division of Laboratories and Research, New York State Department of Health, Albany.

Received for publication, October 2, 1926.

per cent salt solution necessary to make the desired dilution, which is mixed well by shaking. The cholesterinized antigen is diluted as follows: The appropriate amount of the cholesterinized extract is added rapidly to the amount of salt solution necessary to make the desired dilution, and the dilution is mixed well by shaking. In the case of cholesterinized antigens, comparison is always made of dilutions prepared by both slow and rapid mixture of extract and salt solution, and the method is adhered to which gives the more favorable results.

Recently, a portion of the acetone-insoluble antigen was reinforced with 0.4 per cent of cholesterin for some experimental tests. When diluted in the same manner as the routine cholesterinized antigen and compared with it in complement-fixation tests, it gave much weaker results. Although it seemed improbable that antigens containing cholesterin could be satisfactorily suspended in salt solution after drying, an attempt was made to dilute the antigen by Bordet's method in the same manner as the plain acetone-insoluble antigen. There evidently was some precipitation of the cholesterin, as the resulting suspension was somewhat grainy, but the dilution prepared by this method gave decidedly stronger results than did the dilution prepared by adding the fluid extract to the salt solution without evaporation.

It then occurred to us that certain cholesterinized extracts, which had been found less sensitive than our routine cholesterinized extract, might, by this method of dilution by evaporation, have their antigenic properties sufficiently increased to be satisfactory for routine use. A cholesterinized extract (W 23) was selected, which had previously been tested parallel with the routine cholesterinized antigen (W 17). Out of 195 complement-fixation tests, the results had agreed in only 139. Of the 56 tests which had disagreed, 52 had been stronger with the routine antigen, the differences in about half these cases having been marked. This insensitive W 23 antigen was then diluted by Bordet's method of evaporation, with gratifying results. Five hundred and twenty-six comparative tests were made with it and the routine cholesterinized antigen, and in 438 tests, the results agreed. Of the 88 tests that disagreed, 48 were slightly stronger with the routine antigen, and 40 were slightly stronger with the W 23 antigen diluted by Bordet's method. In no case was the difference in the degree of fixation obtained, marked. Thus, Bordet's method of dilution renders this antigen satisfactory for use in routine complement-fixation tests for syphilis, whereas, when diluted by the usual method, it had proved too insensitive.

To determine whether the increased cloudiness of the suspension was the factor responsible for the increase in sensitivity, a small series of comparative tests was made with a dilution prepared by Bordet's method and with a cloudy dilution prepared by adding salt solution slowly to the unevaporated extract. Approximately one-fourth of the serums tested showed differences in the degree of fixation obtained with these two cloudy dilutions, the fixation being greater with the dilution prepared by Bordet's method in all the cases where differences appeared. The sensitivity of this antigen seems to depend to some extent, therefore, upon the method of its dispersion in salt solution.

Another cholesterinized extract, on the other hand, was found to give equally satisfactory results with cloudy solutions prepared by Bordet's method and by adding salt solution slowly to the unevaporated extract. Our routine cholesterinized extract gives equally satisfactory results with cloudy dilutions prepared by the two methods and with a clear dilution prepared by adding the extract rapidly to salt solution. The optimum dilution of the antigen is lower, however, when cloudy solutions are used, the 1:50 dilution being the optimum with cloudy solutions and the 1:100 dilution, the optimum with a clear solution.

These findings are at variance with the observations of Griffith and Scott,[3] who state that the Bordet method of dilution applied to cholesterinized extract produces a suspension of diminished instead of improved efficacy. This conclusion might be reached if the titer of the antigen were taken as the measure of its effectiveness, since a rather anomalous fact noted by us was that there appeared to be no parallel between the titer of the antigen and its sensitivity. Antigen W 23 had a higher titer when diluted by adding the extract directly to the salt solution, but was decidedly more sensitive when diluted by Bordet's method of evaporation. When diluted directly, this antigen fixed completely in 0.1 c.c. of a 1:400 dilution and, when diluted by Bordet's method, it gave complete fixation with 0.1 c.c. in a dilution no higher than 1:100. The optimum dilution for the evaporated extract was found to be 1:50 and for the extract diluted directly, 1:100, the most sensitive results being obtained with this 1:50 dilution prepared by Bordet's method.

Other extracts which have been found too insensitive for use are to be tested by diluting the evaporated extract, and it is hoped and believed that these antigens will likewise be found satisfactory by this method.

A trial of this method for diluting antigens is recommended, since, with care in thoroughly suspending the dried residuum, a perfectly uniform dilution can always be made. On the other hand, when antigen is diluted by adding extract directly to salt solution or vice versa, there are bound to be slight variations, according to individual differences, in the degree of rapidity with which the mixture is made.

REFERENCES

[1]Bordet, J., and Ruelens, G.: L'antigene syphilitique de l'Institut Pasteur de Bruxelles, Compt. rend. Soc. de biol., 1919, lxxxii, 880.

[2]Neymann, C. A., and Gager, L. T.: A New Method for Making Wassermann Antigens from Normal Heart Tissue, Jour. Immunol., 1917, ii, 573.

[3]Griffith, F., and Scott, W. M.: Technic of the Wassermann Reaction, Ministry of Health Reports on Public Health and Medical Subjects, 1920, No. 1, p. 7.

A CASE OF POLYCYTHEMIA VERA TREATED WITH PHENYLHYDRAZIN HYDROCHLORIDE WITH SPECIAL REFERENCE TO CHANGES IN BLOOD MORPHOLOGY*

By Hugo O. Altnow, M.D., and James B. Carey, M.D., Minneapolis, Minn.

THE treatment of polycythemia vera by the use of arsenicals[8] and benzol, by splenectomy,[9] venesection and intravenous saline infusion,[10] and even by irradiation in its various forms, has been so unsatisfactory that any new procedure is worthy of serious trial if there is any evidence at all that it might be successful.[11-21]

The hydrochloride of phenylhydrazin was first suggested and used by Eppinger[1] in the treatment of polycythemia vera, following the demonstration by Moravitz and Pratt[2] that an anemia could be produced in animals by its administration. In his report of four cases, Eppinger used the drug in 1 to 5 per cent solution by hypodermic injection subcutaneously in amounts varying from 2 to 12 c.c., beginning with small doses of the 1 per solution and gradually increasing both the strength of the solution and the size of the dose. Reduction in total red cell count and hemoglobin percentage, leucocytosis, jaundice and tenderness of the spleen were noted in all cases.

Taschenburg[3] followed up the work of Eppinger with essentially the same results. He reported in addition to a leucocytosis, the presence of 15 per cent myelocytes and myeloblasts, together with nucleated red cells, when the total count had been reduced to 1,000,000 red cells and the hemoglobin percentage to 30. He used 9.65 gm. of the drug in divided dosage over a period of five and one-half months.

The first reports in this country were by Trevor Owen in 1924[4] and 1925.[5] His use of the drug differed from that of the other authors in that he administered smaller doses, by mouth, over an extended period of time.

None of these men apparently made any observations on the changes occurring in the blood cells themselves, and it is on this account, as well as to place on record another case of polycythemia vera treated with phenylhydrazin hydrochloride, that we are submitting the following report.

Phenylhydrazin has a chemical formula of C_6H_5–NH–NH_2. The hydrochloride has been used in work on human subjects as being the least toxic form. The basic drug becomes oxidized to set free a benzol ring which probably is the actual active agent bringing about the blood destruction. It is a member of the antipyrin group of chemical substances, and also, in its action, bears some relation to quinine.

The case we wish to report is that of a Jewish woman fifty-three years of age, married, with husband and five children all living and well. Her

*From the Medical Division, The Nicollet Clinic, Minneapolis Minn.
Received for publication, October 14, 1926.

family history is negative, and her past history uneventful except for an attack of pleurisy at the age of forty.

During the summer of 1921 she passed tarry stools for a few days and in 1922 she had, apparently, a gastric hemorrhage. In November, 1923, she felt so ill that she sought medical advice at the Mayo Clinic.

The blood examination at that time showed: Hemoglobin, 81 per cent. Red blood cell count, 5,570,000, hematocrit 56 per cent of red blood cells. White blood cells, 5,100. Viscosity, 1:7.4. Whole blood volume, 151 c.c. per kilo.

On the basis of these findings, and a palpable spleen, a diagnosis of polycythemia vera was made.

The patient returned to Rochester in October, 1924, at which time the blood examination showed: Hemoglobin, 84. Red blood cell count, 6,440,000. Viscosity, 1:7.6.*

She presented herself at this Clinic on Nov. 27, 1924, complaining of vertigo, nausea, a sense of internal heat and generalized "neuralgie" pains. Our examination revealed a well-nourished woman, appearing somewhat younger than the stated age of fifty-three, with a high color, congestion of the conjunctiva and slight cyanosis of the mucous membranes. Physical examination revealed the liver edge one and one-half inches below the costal margin and the spleen three inches below the costal margin. The hemoglobin at this time was 89 and the red blood cell count was 6,970,000. From this time until November, 1925, she had extensive irradiation with the x-ray directed to the spleen and long bones, combined with benzol. The lowest blood count during this period was 5,100,000, and there was never at any time any symptomatic relief.

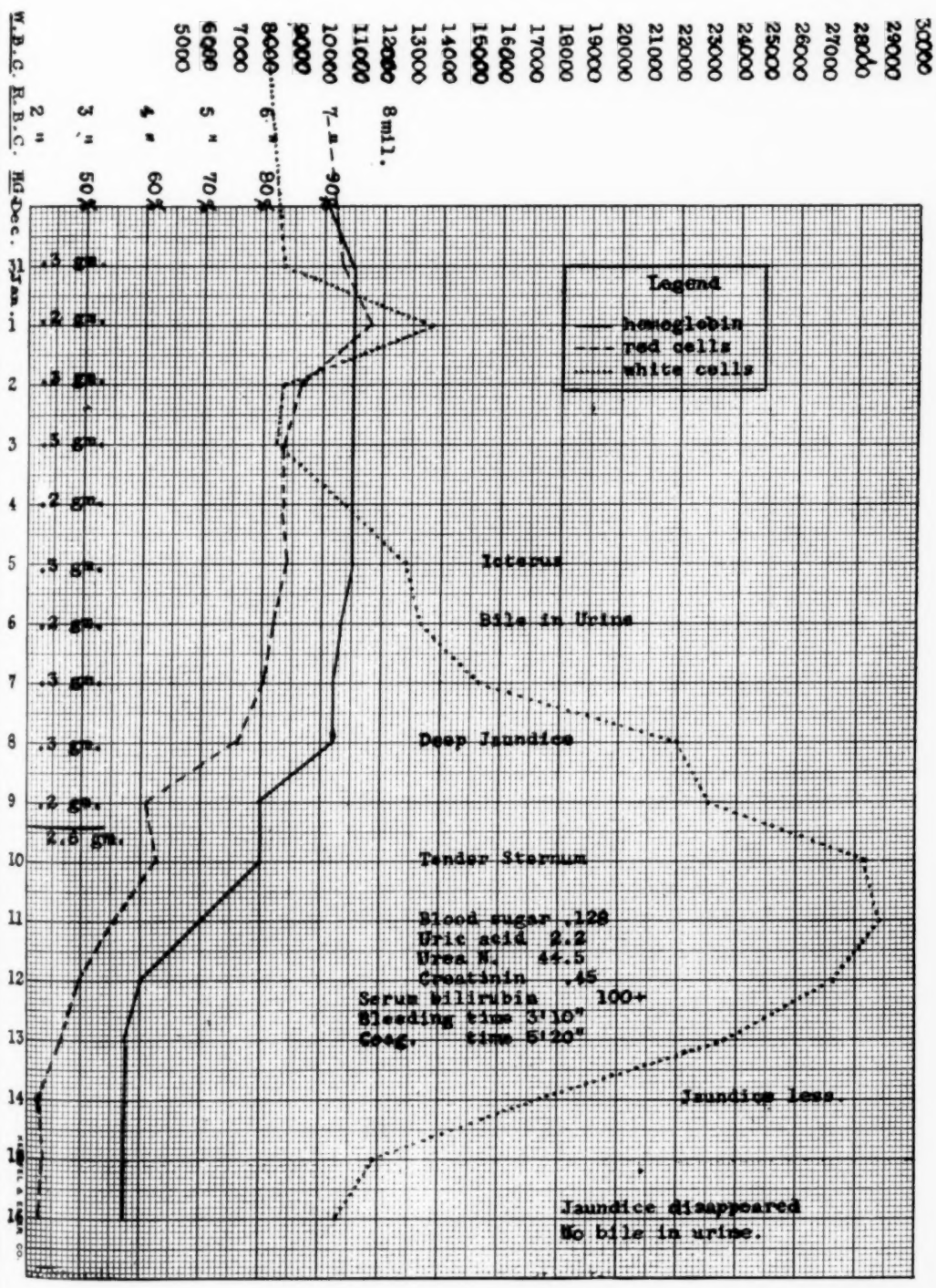
On December 31, 1925, she was hospitalized for treatment by phenylhydrazin hydrochloride. Her blood count on that date showed hemoglobin 95 (Dare); red blood cell count 7,340,000; white blood cell count 8,700. Subsequent blood counts during this treatment are charted (Fig. 1).

She received a total of 2.6 gm. of the drug in 0.1 gm. doses from December 31 to January 10, inclusive. During this time she became heavily jaundiced and the liver and spleen became enlarged and very tender. Her urine and stool contained bile and bile pigments in large amounts. The drug was discontinued on the tenth day of January, but the red cell count continued to drop until the lowest point was reached on the sixteenth. By that time the spleen and liver had receded, the jaundice had cleared up, and the excreta had returned to a normal content of bile and pigment.

On the eleventh day the fasting blood sugar was 0.128, the uric acid was 2.2, the urea nitrogen was 44.5, and the creatinine was 0.45. On this date the icterus index was 100+, according to the technic of Murphy.[6]

The patient was discharged from the hospital on the sixteenth and reported to the office on the twenty-third of January. At that time her hemoglobin was 74. The red blood cells had increased to 3,380,000 and the white cells to 6,100. She reported that she was much more comfortable than

*We are indebted to Dr. Giffin of the Mayo Clinic for these findings and for the privilege of reporting them.


W.B.C. R.B.C. Hg. Dec. Jan.
2 "
3 " 50%
4 " 60%
5 " 70%
6 " 80%
7-8-9 90%
8 mil.
3000
29000
28000
27000
26000
25000
24000
23000
22000
21000
20000
19000
18000
17000
16000
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.3 gm.
.2 gm.
2.5 gm.
Legend
hemoglobin
red cells
white cells
Icterus
Bile in Urine
Deep Jaundice
Tender Sternum
Blood sugar .128
Uric acid 2.2
Urea N. 44.5
Creatinin .45
Serum bilirubin 100+
Bleeding time 3'10"
Coag. time 5'20"
Jaundice less.
Jaundice disappeared
No bile in urine.

Fig. 1.

she had been for many years. The spleen was only about one and one-half inches below the costal margin and the liver was not palpable.

A count on January 29 showed hemoglobin 78, red blood cells 3,680,000 and white blood cells 6,900. She felt so well that it was decided to undertake some dental work which she had long delayed. On Feb. 19, 1926, she reported, after the dental work was completed, feeling very fine and with no complaints. The hemoglobin was then 80, the red blood cells 4,600,000, and the white blood cells 7,750.

On March 22 a blood count was made with the finding of hemoglobin 85, red blood cells 5,590,000. On April 10 the hemoglobin was 83, red blood cells 5,740,000 and white blood cells 9,500, and she was still free from complaints. Physical examination showed a palpable spleen, but otherwise normal findings.

On May 14 she reported at the office complaining of pain and "blisters" on the back and right side. She was found to have a herpes zoster on the right posterior thoracic region at about the level of the ninth vertebra. The blood findings at that time were hemoglobin 88, red blood cells 5,720,000 and white blood cells 6,700. It was planned that we would give her small doses of phenylhydrazin hydrochloride (probably 0.1 gm. per day) as soon as the herpes had subsided.

It is evident from the results obtained in the above case that the drug is a powerful hemolyzing agent, and that the reduction in the total number of red blood cells has been produced by hemolytic destruction. The spleen and liver enlarged and became tender, but according to the figures obtained in the chemical study of the blood at the height of the hemolysis, there was no actual liver damage.

Levi[7] reports a case of polycythemia which had been treated with phenylhydrazin and which came to autopsy, in which a cirrhosis of the liver was found. This case had a total of 7.5 gm. of the drug over a period of eighteen months and died of erysipelas. The autopsy showed a cirrhosis of the liver and enlarged spleen with infarction, coronary sclerosis, ulcers of the stomach and duodenum and a bronchopneumonia, but the history of the case showed that the man had been a heavy beer drinker, which might account in part for the hepatic condition. Also, cirrhosis of the liver has been reported many times as a finding at autopsy in polycythemia cases which had not been treated with phenylhydrazin.

In addition to the enlargement of the liver and spleen, hemolytic activity was further shown in our case by the deep jaundice of skin and sclerotics and the increased amounts of bile and bile pigments found in the excreta (urine and feces). Also, accompanying this very marked destruction of the red blood cells, or even preceding such destruction, there was an increase in the white blood cells, indicative of bone marrow stimulation. This was further predicated by the fact that the stool and urine were both freighted to capacity with bile and bile pigments, which could not occur to such a degree if the blood were only being destroyed, it being possible only because of a concomitant increase in production. There then followed, apparently, a depressant phase, perhaps of bone marrow exhaustion and depletion. Dur-

ing this period the total white count fell rapidly, the jaundice cleared up and the bile products disappeared from the urine and stool. At the same time our observation of the white blood cell picture indicated an effect on those elements which has not, in our survey of the literature, been reported in any other discussion of the subject.

TABLE I

Hg.	R. B. C.	W. B. C.	LYMPHOCYTES	MONOCYTES	POLYS	EOSINOPHILES	BASOPHILES	MYELOCYTES	OBSERVER
155	8,600,000	6,600	22.8	3.0	64.8	2.8	.6		Weber
180	10,520,000	5,660	25.6	1.7	70.7	.3	1.7		Weber
168	9,150,000	16,000	7.7	1.5	80.9	4.6	5.3	few	Von DeCastello
150–160	8,000,000	6,600							
	10,000,000	8,500	16.0	.0	75.0	5.0	.0	4.0	Kuttner
120	11,960,000	14,400	4.0	24.0	71.0	1.0	.0		Umber
135–150	7,900,000	5,300							
	9,200,000	8,700	5.0	11.0	77.0	4.0	.0		Beltz

In view of the fact that we did not interest ourselves in the intensive study of the whole blood until the thirteenth day after the beginning of the treatment, our data are incomplete. However, the striking changes produced make it possible to interpret certain features in terms of action of the drug on the bone marrow and the reticuloendothelial system. Unfortunately no differential count was done before the administration of phenylhydrazin. An idea of the blood picture in erythremia in general may be obtained by examination of Table I, which is a tabulation from E. Parkes Weber[22] of all complete blood counts given on patients with erythremia from the time that the diagnosis of this condition was first made, up to the time of the publication of his monograph in 1921. It will be noted that the combined lymphocyte and monocyte count in only one case (that of Von De Castello) approaches the low level reached in our case. We call attention to this feature to comment more fully on it later.

Furthermore, it may be argued that in our patient permanent reduction in the lymphocyte and monocyte count may have been influenced by the previous irradiation and benzol treatment received before the administration of phenylhydrazin. Minot and Spurling[23] have shown that the lymphocyte is the most sensitive cell to roentgen rays and that the lymphopenia is the last cell change to be adjusted. This is more particularly true when the irradiation has been sufficient to produce leucopenia. However, in both instances, three days following irradiation there was a gradual increase in the lymphocyte count, so that at the end of approximately forty days the total lymphocyte count reached the level that existed before the exposure. In our patient a longer period since the last irradiation had elapsed.

Recognizing these objections and omissions in our data, we shall proceed to analyze the blood counts of our patient as recorded in Fig. 1 and Table II. The most striking feature is probably that of the diminution of the red cell count, that, except for minor fluctuation, was progressive and reached its maximum on the sixteenth day following the beginning of the administration of the drug. Clinical evidence supports the premise that this change was due to destruction of the red cells. Coincident with this and equally impres-

TABLE II

DATE	PLATELETS	RED BLOOD CELLS						WHITE BLOOD CELLS					
		ANISOCYTOSIS	POIKILOCYTOSIS	POLYCHROMASIA	ACHROMIA	NUCLEATED REDS	RETICULATED R. B. C.	LYMPHOCYTES	MONONUCLEARS	POLYS	EOSINOPHILES	BASOPHILES	Resembles P. A. Number of Microcytes. Large.
1/12/26	N	++++	+++	+++	++	12		5.5	4.0	87.	.5	3.0	Basophile cells as found in Leucemia. Numerous broken r. b. c.
1/13/26	N	++++	+++	++	+	0	8.4%	13.0	2.5	83.5	.5	.5	R. B. C. picture still like that of Pernicious Anemia.
1/16/26	N	++++	++++	++	+	0	4.7%	11.5	1.5	81.5	1.5	4.0	Many macrocytes and microcytes and pale red cells. Numerous "bizarre" lymphocytes.
1/23/26	+1	++	+	+	++	0	2.4	7.0	3.0	87.5	.0	2.5	Few microcytes and macrocytes.
1/29/26	+2	++	+	0	++	0		5.5	1.0	92.0	1.0	1.0	Occas. microcyte and poikilocyte. Fairly normal r. b. c's.
2/19/26	+2	++	++	0	++	0	.4	9.0	1.0	87.5	.5	2.0	Occas. microcytes.
4/10/26	N	++	+	0	++	0		12.0	7.0	79.5	.0	1.5	Less poikilocytosis. Otherwise same.
5/14/26	+2	++	+	0	++	0		9.0	6.5	83.0	2.0	.5	

sive was the rising white blood cell count that reached its maximum from the tenth to the twelfth day. That this was due to marked stimulation of the marrow was made further evident by the presence of nucleated red cells in fairly large numbers on the twelfth day and 8.4 per cent reticulocytes on the following day. From this point there was a progressive decrease in the reticulocytes and on Jan. 23, 1926, the count was 2.4 per cent, a figure which is slightly above normal. The smears taken on the twelfth, thirteenth and sixteenth days showed very marked anisocytosis with numerous macrocytes and microcytes (especially the latter), large pale red cells, fragmented cells and "ring" forms. In many respects the appearance of the smear was identical with that of pernicious anemia in a hemolytic crisis.

On the twenty-third day there was a marked reduction in anisocytosis and poikilocytosis. This remained so on subsequent examinations and the last examination showed moderate anisocytosis, few poikilocytes and moderate achromia, on the whole more suggestive of simple chronic anemia. Platelet counts were not made, but the number of platelets was estimated, five to ten platelets per oil immersion field being considered as the normal standard. During the stage of active bone marrow activity when reticulocytes and white blood cells were increased, the platelets were apparently not increased. Later, when there was a decrease in these elements, there was a moderate increase in the number of platelets.

The effect of the drug on the white blood cell picture is more difficult to analyze. It is perhaps most easily comprehended if we consider separately the effect produced on the cells of the lymphocytic and myeloid series. It is generally accepted that the lymphocytes are produced in the lymph glands and the reticuloendothelial tissues of the spleen, liver, bone marrow, pharynx, and other reticuloendothelial structures. This is in conformity with the opinion of Weidenreich.[24] There is more uncertainty as to the origin of the monocyte or mononuclear cell, but the same author states that it can differentiate itself from these same structures. McJunkin[25] concludes that there are two types of mononuclear phagocytes present in human blood, namely, (1) monocytes, which are probably produced in the bone marrow and spleen, since they are found only in these fixed tissues, and (2) lymphendotheliocytes, arising from the lymphatic reticuloendothelium. Acting on these sources of information, it is reasonable to conclude that the source of production of cells of the lymphocytic series (lymphocytes and mononuclears) is in the reticuloendothelial system.

The statement that the polymorphonuclear leucocyte is chiefly produced in the bone marrow does not require substantiation, since this is the generally accepted view. These cells, together with the polymorphonuclear eosinophile, constitute the myeloid cells of the peripheral blood. Weidenreich considers the bone marrow the usual site of origin of the eosinophile cells.[24]

The basophile cell must be considered as a special type of cell, neither lymphoid nor myeloid in origin. Weidenreich[24] (ibid., p. 256), Pappenheim and Proscher (quoted by Weidenreich) consider the basophile or mast cell to be a degenerating form of the nongranular lymphocyte. This offers a satis-

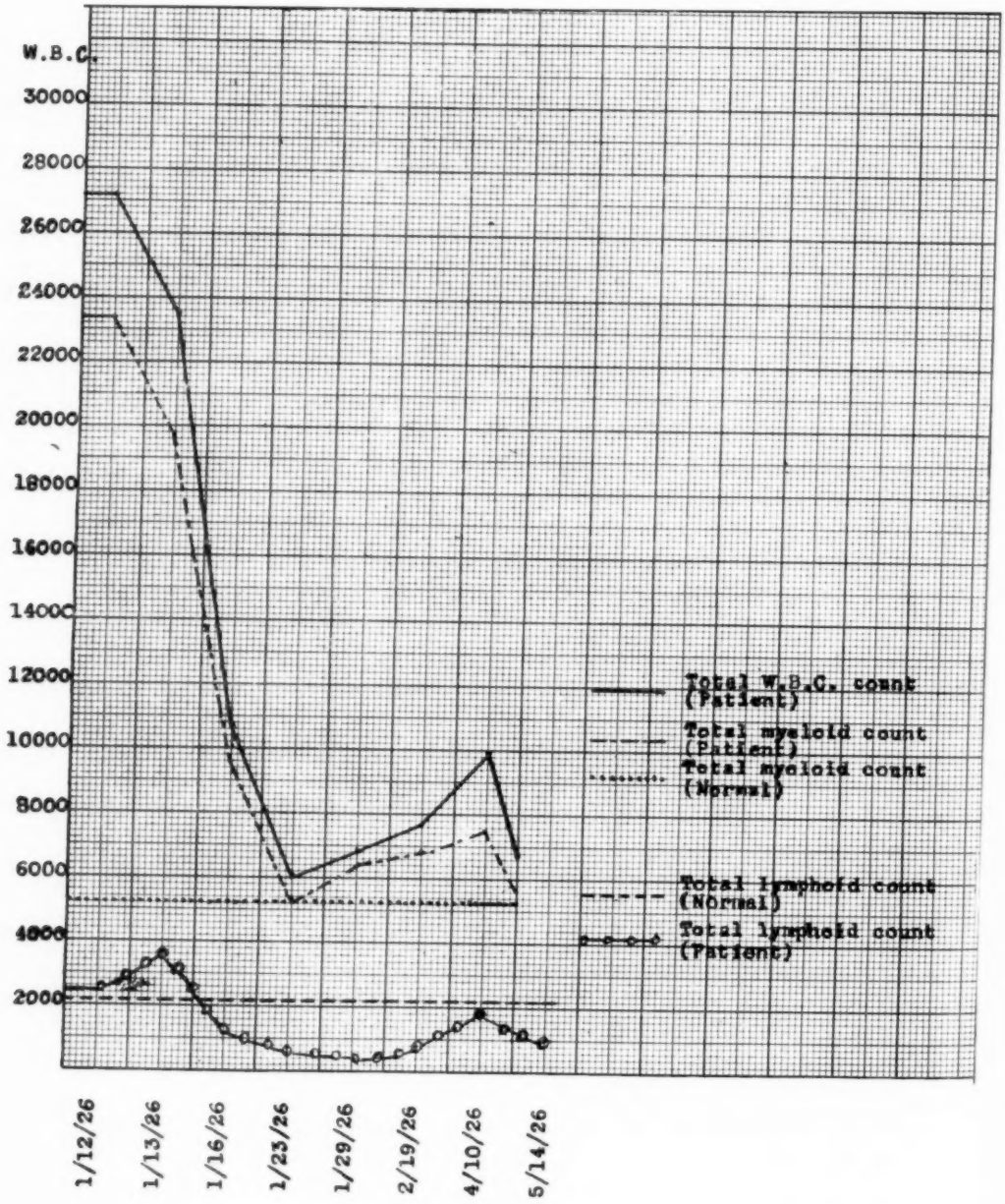
W.B.C.
30000
28000
26000
24000
22000
20000
18000
16000
14000
12000
10000
8000
6000
4000
2000
Total W.B.C. count (Patient)
Total myeloid count (Patient)
Total myeloid count (Normal)
Total lymphoid count (Normal)
Total lymphoid count (Patient)
1/12/26
1/13/26
1/16/26
1/23/26
1/29/26
2/19/26
4/10/26
5/14/26

Fig. 2.

factory explanation for their appearance in large numbers in myelogenous leucemia where these elements are disappearing from the circulation.

We consider it necessary to establish the above facts as to the origin of the white blood cells before proceeding with our comment on the action of phenylhydrazin on the white blood cell picture of the subject of our case report. This action is portrayed in the behavior of the total absolute count of the cells of the myeloid and lymphoid series, as shown in Fig. 2.

The curve of the myeloid cells (polymorphonuclear neutrophiles and eosinophiles) parallels very closely the total white blood count. At no time does it fall below 5300 (total polymorphonuclear neutrophile and eosinophile count in normal blood on the basis of white blood cell count of 7500). The total lymphoid count (lymphocytes and mononuclears) apparently does not share in the initial increase, as do the myeloid cells, and shows approximately a 60 per cent increase as compared with an approximate 700 per cent increase in the latter on the thirteenth day. There is also a correspondingly greater decrease that is sustained for a longer period. At its maximum (January 29) it is less than 20 per cent of the normal total lymphoid count and at no time after the initial rise does it attain the normal figure (2100 on the basis of 7500 white blood cells in a normal individual). Judging from this, it appears that the phenylhydrazin exerts a more depressant effect on the reticuloendothelial structure than it does on the other hemopoietic tissues. It is even doubtful as to whether it has any such action on the latter. On the other hand, it seems to be one of stimulation and the red blood cell count is driven down, not because of diminished red blood cell formation, but because destruction is proportionately much greater than production. That the drug should exhibit a depressant or toxic effect on the reticuloendothelial system is not remarkable, when we take into consideration the fact that according to the newer knowledge, hemolysis and the formation of bilirubin take place at the same site.

The basophile count is from two to eight times above the normal in all but two of the eight counts. If we accept the hypothesis of Weidenreich that they are a degenerative type of the nongranular lymphocytes and a cell undergoing retrograde metamorphosis, their presence in increased numbers is easily explained when, as in this case, the nongranular cells were diminishing in number. As additional support to this hypothesis we may also refer to Gruner[26] who states that "The lymphocyte of the blood stream, for instance, may undergo mucoid degeneration under the influence of certain toxins (staphylotoxin, phynolysin) and constitute the mast cell of the blood stream." Other substances mentioned by him producing increased numbers of mast cells are pyrodin, hemialbumose, colchicine, tuberculin, milk and cancer extracts. It is therefore quite possible that phenylhydrazin brings about an increase in these cells in a similar manner.

CONCLUSIONS

1. It is possible with the proper dosage and administration of phenylhydrazin hydrochloride to bring about complete amelioration of symptoms of the disease.

2. The use of the drug reduces the total red blood cell content of the blood, and can even produce an anemia.

3. The reduction in the red blood cell count is an actual hemolytic destruction of these elements of the blood, evidenced by clinical and morphologic studies.

4. There is also apparent a considerable stimulation of the bone marrow, as seen in the increase in total white cell count and number of nucleated red cells and reticuloeytes.

5. Administration of the drug in this case produced a marked reduction of the absolute lymphoid count, either by destruction of these cells or by a depressive effect on the reticuloendothelial structures inhibiting their formation.

NOTE: The subject of this report has been under constant observation since May, 1926. She was symptom free until November, at which time she required a course of the drug (0.1 gm. daily for two weeks), which was repeated in December. Now, in March, 1927, she is comfortable again.

REFERENCES

[1]Eppinger and Kloss: Therap. Monatsh., 1918, xxxii, 322.
[2]Moravitz and Pratt: München. Med. Wchnschr., 1908, iv, 35, 817.
[3]Taschenburg: Deutsch. Med. Wchnschr., 1921, xlvii, 27, 774.
[4]Owen: Bull. Johns Hopkins Hosp., 1924, xxxv, 258.
[5]Owen: Jour. Am. Med. Assn., 1925, lxxxv, 26, 2027.
[6]Murphy: Bost. Med. and Surg. Jour., 1926, cxciv, 297.
[7]Levi: Ztschr. f. klin. Med., 1924, c, 6, 777.
[8]Hofman: Dermat. Ztschr., 1924, xli, 348.
[9]Sauer: Deutsch. Med. Wchnschr., 1924, l, 1635.
[10]Betz: Kaufman: Klin. Wchnschr., 1924, iii, 1841.
[11]Rydgaard: Hospitals Tiedende (Copenhagen), 1921, lxiv, 24, 379.
[12]Bottan: Deutsch. Med. Wchnschr., 1921, xlvii, 27, 773.
[13]Schnitze: Med. klin., 1923, xix, 458.
[14]Mosenthal: Med. Klin., 1923, xix, 1158.
[15]Gaisboch: Klin. Wchnschr., 1923, ii, 669.
[16]Strasburger: Therap. Halbmonatsch., 1921, xxxv, 35, 777.
[17]Pendergrass: Am. Jour. Med. Sc., 1921, clxi, 5, 723.
[18]Wells: Jour. Exper. Med., 1908, x, 457.
[19]Underhill-Kleiner: Jour. Biol. Chem., 1908, iv, 165.
[20]Underhill: Jour. Biol. Chem., 1914, xvii, 295.
[21]Bodansby: Jour. Biol. Chem., 1924, lviii, 799.
[22]Weber, E. Parkes: Polycythemia, Erythrocytosis and Erythrecemia, London, H. K. Lewis & Co., Ltd., 1921.
[23]Minot-Spurling: Am. Jour. Med. Sc., 1924, clxvii, 2, 215-241.
[24]Weidenreich: Die Leucocyten und Verwandelte Zellformen, Wiesbaden, J. F. Bergman, 1911.
[25]McJunkin: Arch. Int. Med., 1925, xxxvi, 5, 799.
[26]Gruner: The Biology of the Blood Cells, Wm. Wood & Co., New York, 1914.

A SIMPLE BLOOD CULTURE TECHNIC*

By Perry J. Manheims, M.D., and Irene S. McGrath, A.B., New York City

THE use of an anticoagulant in taking blood cultures is not new, and various methods have been devised of simplifying the complicated procedures which are in more or less common use. Epstein[1] suggested the use of ammonium oxalate to prevent coagulation so that elaborate media at the bedside could be dispensed with. Chantemesse[2] and later Garbat[3] in their work on blood cultures in typhoid fever, substituted sodium citrate for sodium oxalate. Ryttenberg[4] used an ammonium oxalate and sodium chloride solution in taking blood cultures while Lintz[5] employed a 1 per cent sodium citrate in normal saline solution or a 0.2 per cent ammonium oxalate in 0.6 per cent saline solution. Reichard[6] advocates powdered sodium citrate as an anticoagulant.†

The following technic has been employed in this laboratory for more than two years because of its simplicity and efficiency. One hundred c.c. of a 2 per cent sodium citrate solution is sterilized and kept as a stock solution. When a blood culture is to be made, 1½ c.c. of this is pipetted into a sterile cotton stoppered test tube (size 6x1) and boiled for a few minutes over an open Bunsen flame thus insuring sterilization of the citrate solution and at the same time making doubly certain of the sterilization of the tube. Approximately 1 c.c. of citrate solution is left in the tube which is then allowed to cool. It is only necessary to take to the bedside, the test tube containing the sterile sodium citrate solution, and a package in which are a sterile 10 c.c. Luer syringe and 2 needles (18-20 gauge).

The patient's arm is prepared in the manner usual for blood culture, 9 c.c. of blood are withdrawn and well mixed with the 1 c.c. of citrate solution in the tube, thus making a 0.2 per cent sodium citrate solution in blood, a percentage which is sufficient to prevent coagulation and which at the same time does not inhibit bacterial growth. The citrated blood is then taken to the laboratory and cultures made on suitable media. This is done as soon as possible after the blood is withdrawn but an hour's delay has apparently not interfered with bacterial growth.

For approximately six months the citrate method was paralleled with the old method of taking the media to the bedside. During this time there were 61 negative and 10 positive blood cultures and in every instance the results were the same with both methods. Since the citrate method has been adopted as routine our results have been as follows:

*From the Achelis Laboratory of the Lenox Hill Hospital, New York City.
Received for publication, October 3, 1926.

†Since the inauguration of this method, Colebrook and Storer[7] have shown that sodium citrate *inhibits* the bactericidal power of blood, and that this is more marked in freshly shed blood than in blood serum.

Total number of Blood Cultures	535
Negative Cultures	457
Positive Cultures	78
B. Typhoid	17
Staph. Albus	13
Staph. Aureus	10
Strep. Viridans	10
Strep. Hemolyticus	8
Pneumococcus	7
Bacillus Coli	6
Nonhemolytic Strep.	5
Diphtheroid B.	1
Meningococcus	1

The advantages of the citrate method of taking blood cultures are:

1. This method is much simpler than those previously employed in which the media was carried to the bedside. It eliminates the difficulty of keeping the melted agar at the proper temperature while the blood is being taken.

2. The results are as good if not better than with the older complicated methods.

3. There is less chance of contamination because the cultures are made without haste and with proper facilities in the laboratory and not at the bedside.

4. There is less psychic upset to emotional patients.

CONCLUSIONS

The addition of 2 per cent sterile sodium citrate solution in sufficient quantity to prevent the coagulation of blood does not inhibit the growth of pathogenic organisms in this blood and provides a simple and satisfactory method of taking routine blood cultures.

REFERENCES

[1]Epstein: Am. Jour. Med. Sc. New Series, September, 1907, cxxxiv, No. 3.
[2]Chantemesse: Quoted by Ryttenberg.
[3]Garbat, A. L.: Personal Communication.
[4]Ryttenberg, Chas.: Jour. Med. Research, New Series, January, 1909, xv, No. 1.
[5]Lintz, Wm.: New York Med. Jour., April 3, 1915, ci. No. 14.
[6]Reichard, J. D.: The Military Surgeon, April, 1926, lviii, No. 4.
[7]Colebrook, L., and Storer, E. J.: Brit. Jour. Exper. Path. 1924, v, 47.

STUDIES OF QUANTITATIVE BLOOD-SUGAR ESTIMATION*

Various Methods Compared with the Author's Micro-Folin-Wu Method

By Thomas Luther Byrd, M.D., Milwaukee, Wis.

DURING the past few years, the chemical analysis of blood, for its various constituents, has gained an undisputed field in medical research. The data obtained by quantitative blood-sugar estimations are not only of value, from a standpoint of diagnoses, in cases presenting glycosuria, and other symptoms complex, but furnish a better scientific knowledge as to the severity and the best rationale to observe in treating cases of both hyperglycemia and hypoglycemia, irrespective of the conditions causing these symptoms. As the standard methods of blood-sugar determinations now in use require 2 c.c. or more of blood for a single analysis, which necessitates a venipuncture, there are great numbers of patients, including infants, small children, and obese individuals, who cannot share in these advantages. Sugar tolerance tests are not done in many instances where they are indicated, because of the obstacles encountered by both operator and patient in doing a series of venipunctures at frequent intervals.

One year ago I devised a means[1] requiring a minimum quantity of blood, applicable to all patients indicating the need of a quantitative blood-sugar estimation. Comparative studies were made, with the Folin-Wu and the Micro-Folin-Wu methods[2] and with the latter method alone, on a sufficient number of specimens of blood to demonstrate that the latter is just as reliable as the former method, regardless of the sugar content of the specimens analyzed. These comparisons were made on a practical basis; that is, the unknown of one technic was read as accurately as possible against its respective standard, the standards then changed, and the unknown of the other technic brought to the same figure as the former, and they invariably coincided.

Further comparative studies were made on fifty specimens of blood, from both nondiabetic and diabetic patients, with the Benedict modification of the Lewis-Benedict, Myers-Baily, Folin-Wu and the Micro-Folin-Wu methods. The three former methods were chosen because they are the standard methods commonly used as routine in the majority of clinical laboratories, with slight modifications in some instances. The results are given in the accompanying tables.

TECHNIC

The original technics were strictly adhered to in every detail. The standard of the Benedict modification of the Lewis-Benedict method was pre-

*From the Laboratory of the Sacred Heart Sanitarium and St. Mary's Hill, Milwaukee, Wis.

Received for publication Oct. 29, 1926.

TABLE I

COMPARATIVE ANALYSES OF BLOOD SPECIMENS FROM TWENTY-FIVE NONDIABETIC PATIENTS. EXPRESSED IN MILLIGRAMS PER 100 C.C. OF BLOOD

NO.	NAME	FOLIN-WU METHOD USING 2 C.C. BL.	MICRO-FOLIN-WU METHOD USING 0.1 C.C. BL.	BENEDICT MOD. LEWIS-BENEDICT METHOD USING 2 C.C. BL.	MYERS-BAILY METHOD USING 2 C.C. BL.
1	Mrs. F. W. G.	095.2 mg.	094.3 mg.	108.1 mg.	102.0 mg.
2	Mrs. A. A.	090.9 "	090.9 "	090.9 "	100.0 "
3	Miss A. E.	111.0 "	111.0 "	111.0 "	111.0 "
4	Mrs. R. E. K.	095.2 "	100.0 "	107.5 "	102.0 "
5	Mrs. E. W. McD.	100.0 "	100.0 "	100.0 "	100.0 "
6	Mrs. J. C.	083.3 "	083.3 "	090.9 "	083.3 "
7	Mrs. H. O. F.	111.0 "	111.0 "	111.0 "	111.0 "
8	Mrs. H. G.	092.5 "	090.2 "	098.3 "	095.2 "
9	Miss M. E.	100.0 "	100.0 "	120.0 "	111.0 "
10	Mrs. E. W. K.	087.6 "	085.7 "	111.0 "	097.5 "
11	Mr. L. O.	103.6 "	103.6 "	115.3 "	099.2 "
12	Mr. J. O. L.	103.6 "	100.0 "	107.5 "	100.0 "
13	Mr. M. G.	107.1 "	107.1 "	125.0 "	122.5 "
14	Mrs. S. M.	100.0 "	100.0 "	111.0 "	111.0 "
15	Mrs. N. O. L.	084.5 "	083.9 "	097.5 "	101.9 "
16	Mr. P. S.	097.5 "	097.5 "	098.4 "	093.2 "
17	Mr. J. P. S.	101.7 "	101.7 "	106.8 "	110.1 "
18	Mr. J. P.	095.2 "	097.5 "	108.1 "	093.9 "
19	Miss O. F.	092.3 "	092.7 "	100.8 "	092.3 "
20	Mrs. J. A.	092.3 "	095.2 "	101.7 "	096.8 "
21	Mr. D. F. G.	096.8 "	096.8 "	112.1 "	107.1 "
22	Miss H. G.	094.7 "	094.5 "	106.2 "	100.8 "
23	Mrs. L. M.	101.7 "	101.7 "	104.3 "	103.5 "
24	Mr. A. T. O.	090.9 "	090.9 "	100.0 "	100.0 "
25	Mr. J. J. C.	097.5 "	098.3 "	101.7 "	099.2 "

A venipuncture was indicated in each instance for other blood tests, and enough extra blood was obtained for sugar estimations, and 0.1 c.c. was collected from the finger tip, at the same time, with the blood diluting pipette for the Micro-Folin-Wu method.

pared by dissolving 0.64 mg. of pure anhydrous dextrose in 4 c.c. of a saturated solution of benzoic acid, instead of using a picramic acid solution for a standard, which is customary in most laboratories employing this technic. All specimens of blood were drawn after a twelve-hour fasting, by a venipuncture (potassium oxalate used as an anticoagulant), and from a pin prick in the finger, with the blood-diluting pipette for the micromethod. The standards and filtrates from each respective technic were boiled simultaneously in the same water-bath, the Folin-Wu tubes were removed after six miuntes, and the other tubes allowed to boil seventeen minutes; the technics terminated in the usual way. A standard (Eimer and Amend) biologic colorimeter was used, the cups measuring 15 c.c. with 2 cm. diameter, the latter being of more importance than the capacity in regards to small quantities. The standards were set at the No. 10 on the colorimeter in each instance because of the small amount (6.25 c.c.) of the micromethod. Three independent readings were made on each unknown, added together, divided by three, the means taken as the figure to compute the values. For example, three readings of an unknown are 6.7, 7, and 6.9; added = 20.6; ÷ 3 = 6.86, the means. The standard reading 10 × 200 (strong Folin-Wu standard) = 2,000; ÷ 6.86 = 291.5 mg. of sugar per 100 c.c. of blood. This will explain the odd figures in the tabulated results.

TABLE II

COMPARATIVE ANALYSES OF TWENTY-FIVE BLOOD SPECIMENS COLLECTED FROM DIABETIC PATIENTS, A NUMBER UNDER INSULIN TREATMENT. EXPRESSED IN MILLIGRAMS PER 100 C.C. OF BLOOD

NO.	NAME	FOLIN-WU METHOD USING 2 C.C. BL.	MICRO-FOLIN-WU METHOD USING 0.1 C.C. BL.	BENEDICT MOD. LEWIS-BENEDICT METHOD USING 2 C.C. BL.	MYERS-BAILY METHOD USING 2 C.C. BL.
1	Mrs. H. B.	166.6 mg.	165.5 mg.	172.4 mg.	179.0 mg.
2	Mr. A. F. (a)	201.6 "	201.6 "	214.3 "	203.4 "
3	" " " (b)	171.5 "	172.7 "	172.1 "	171.5 "
4	" " " (c)	490.2 "	487.8 "	387.6 "	363.8 "
5	" " " (d)	666.6 "	666.6 "	500.0 "	428.5 "
6	Mr. W. W.	155.0 "	155.0 "	157.9 "	168.1 "
7	Mr. J. C. G.	140.3 "	141.1 "	153.8 "	142.8 "
8	Miss E. A.	131.9 "	132.6 "	141.7 "	137.9 "
9	Mrs. D. McG. (a)	414.0 "	414.0 "	400.0 "	333.3 "
10	" " " (b)	230.0 "	237.8 "	250.0 "	226.3 "
11	Mrs. S. R.	223.8 "	222.2 "	236.4 "	240.8 "
12	Mrs. J. J. P.	143.6 "	140.8 "	138.8 "	140.8 "
13	Mrs. S. S. (a)	317.4 "	333.3 "	333.3 "	333.3 "
14	" " " (b)	206.9 "	212.4 "	222.2 "	218.1 "
15	" " " (c)	177.7 "	180.5 "	200.0 "	181.8 "
16	Mr. F. H. (a)	153.8 "	153.8 "	166.6 "	166.6 "
17	" " " (b)	141.2 "	147.2 "	146.4 "	135.3 "
18	Mr. H. F. D.	106.1 "	108.2 "	107.1 "	106.2 "
19	Mrs. C. H. G.	301.4 "	301.4 "	322.5 "	312.5 "
20	Miss R. C. (a)	156.7 "	158.4 "	139.6 "	150.1 "
21	" " " (b)	134.0 "	135.1 "	141.2 "	136.4 "
22	Mrs. A. C. (a)	292.8 "	291.5 "	297.6 "	291.5 "
23	" " " (b)	276.4 "	277.7 "	280.8 "	295.2 "
24	Mrs. K. C.	344.8 "	344.8 "	375.5 "	341.2 "
25	Mr. H. G.	188.6 "	188.6 "	192.3 "	181.8 "

A venipuncture was done in each instance, and 0.1 c.c. was obtained at the same time from the finger tip with the blood diluting pipette.

METHOD OF PRESERVING HEMOLYZED BLOOD

Hemolyzed blood (1 volume of blood plus 7 volumes of distilled water) in any receptacle, or in the blood-diluting pipette as in the Folin-Wu and the Micro-Folin-Wu methods, will maintain the sugar content nine to twelve hours at room temperature, which is sufficient where laboratory facilities are convenient, but not adequate when specimens are drawn at inconvenient hours for analyses or for mailing purposes. To overcome this difficulty it was necessary to find a preservative that could be added to distilled water, which would not interfere with hemolysis or alter the sugar content of the specimens to be analyzed. Several chemicals were tried and 1-400 formaldehyde solution (1 c.c. of commercial 40 per cent formaldehyde in 399 c.c. of distilled water) gave the desired medium. A number of oxalated specimens of blood were hemolyzed with this solution, a portion analyzed immediately, and other similar portions let stand at both room (75 to 85° F.) and incubator (37.5° C.) temperatures for periods of one, two, and three weeks respectively, and several samples hemolyzed in this manner in test tubes and the blood-diluting pipettes were sent by mail to the Mayo Clinic, Rochester, Minn., (285 mi.) and the University Hospital, Augusta, Ga., (1,000 mi.) without deterioration. The small differences in results are attributed to technical errors and minor changes in the sugar standards rather than the preservative being at

TABLE III

GIVEN TIME BLOOD SPECIMENS HEMOLYZED WITH 1-400 (40 PER CENT) FORMALDEHYDE SOLUTION, WILL REMAIN PRESERVED AT ROOM (75-85° F.) AND INCUBATOR (37.5° C.) TEMPERATURES. EX. IN MILLIGRAMS PER 100 C.C. OF BLOOD

BLOOD SPECIMENS NO.	1	2	3	4	
Folin-Wu method, using 2 c.c. of bl.	667		102	293	When taken
Micro-Folin-Wu method using 0.1 c.c. bl.	667	411	105	292	When taken
Benedict Modification of Lewis-Benedict Method, using 2 c.c. blood	500		100	298	When taken
				303	2 wks. at room temp.
				286	2 wks. at incu. temp.
Myers-Baily Method, using 2 c.c. of Blood	426		101	292	When taken
				292	2 wks. at room temp.
				292	2 wks. at incu. temp.
Folin-Wu Method, using 8 c.c. of hemolyzed blood, equivalent to 1 c.c. of whole blood, precipitated, filtered, yielding 2 to 5 c.c. of blood filtrate.	667				1 wk. at room temp.
	667				1 wk. at incu. temp.
		408			When taken
		420			2 wks. at room temp.
		412			2 wks. at incu. temp.
				103	3 wks. at room temp.
				102	3 wks. at incu. temp.
Folin-Wu Method, using 4 c.c. of hemolyzed blood equivalent to 0.5 c.c. of whole blood precipitated, centrifugated, yielding 2.5 to 3.5 c.c. of blood filtrate.	667				1 wk. at room temp.
	667				1 wk. at incu. temp.
		408			When taken
		427			2 wks. at room temp.
		420			2 wks. at incu. temp.
				102	3 wks. at room temp.
				102	3 wks. at incu. temp.
Micro-Folin-Wu method, using 0.8 c.c. of hemolyzed blood equivalent to 0.1 c.c. of whole blood, precipitated, centrifugated, yielding 0.5 to 0.7 c.c. of blood filtrate.	667				1 wk. at room temp.
	667				1 wk. at incu. temp.
		411			When taken
		426			2 wks. at room temp.
		432			2 wks. at incu. temp.
				101	3 wks. at room temp.
				102	3 wks. at incu. temp.

These hemolyzed specimens were placed in unsterile 100 c.c. Erlenmeyer flasks, and corked with rubber stoppers.

TABLE IV

COMPARATIVE ANALYSES OF BLOOD SPECIMENS HEMOLYZED WITH 1-400 FORMALDEHYDE SOLUTION AND SENT TO OTHER LABORATORIES BY MAIL

BLOOD SPEC. DATE	NO. 1	FOLIN-WU METHOD, USING 8 C.C. OF HEM. BLOOD EQUIVALENT TO 1 C.C. OF WHOLE BLOOD	FOLIN-WU METHOD, USING 4 C.C. OF HEM. BLOOD EQUIVALENT TO 0.5 C.C. OF WHOLE BLOOD	MICRO-FOLIN-WU METHOD, USING 0.8 C.C. OF HEM. BLOOD EQUIVALENT TO 0.1 C.C. OF WHOLE BLOOD	MICRO-FOLIN-WU METHOD, USING 0.1 C.C. OF BLOOD COLLECTED WITH BLOOD DIL. PIPETTE
1-28-25	When taken	276 mg.	282 mg.	282 mg.	278 mg.
1-31-25	Univ. Hosp., Augusta, Ga. (1,000 mi.)	267 "	250 "	250 "	267 "
2-27-25	Mayo Clin., Rochester, Minnesota. (285 mi.)	288 "	288 "	288 "	293 "
	No. 2				
2-26-25	When taken	214 "	215 "	213 "	214 "
3- 5-25	Univ. Hosp., Augusta, Ga. (1,000 mi.)	broken	broken	broken	214 "

Note—Specimen No. 1 was analyzed at the Mayo Clinic one month after it was received.

fault. There are four possibilities of a Folin-Wu analysis, of specimens preserved in this way; 2 c.c. of oxalated blood plus 14 c.c. of 1-400 formaldehyde solution furnish more than enough for three analyses, using 8 c.c., 4 c.c., and 0.8 c.c. of the hemolyzed blood, these being equivalent to 1 c.c., 0.5 c.c., and 0.1 c.c. of whole blood, and precipitated accordingly. The fourth applies to 0.1 c.c. of blood collected with the blood-diluting pipette, using 1-400 formaldehyde solution as a diluent. Any of the above quantities of blood, diluted properly (1 to 7 by volume), placed in a suitable container and sealed, or the pipette with a rubber band placed over the ends, can be sent any reasonable distance to a laboratory to be analyzed. Specimens for the Benedict modification of the Lewis-Benedict and the Myers-Baily methods can be preserved likewise, by diluting 2 c.c. of blood, in proportion to each technic.

SUMMARY AND CONCLUSION

These comparisons were made with no intentions of placing discredit on any method used in this study, but to give further evidence that the Micro-Folin-Wu method, using 0.1 c.c. of blood, is just as reliable as any method now in use, irrespective of the quantity of blood necessary for respective technics and regardless of the sugar content of the specimens analyzed. It can be applied to any case indicating the need of a quantitative blood-sugar estimation, including infants, small children, and obese individuals. Its advantages are obvious.

A method of preserving hemolyzed specimens of blood, giving four possibilities of a Folin-Wu analysis; making it feasible to collect and dilute specimens of blood with the blood-diluting pipette and transport any distance, in case laboratory facilities are not convenient.

REFERENCES

1(a) Byrd, T. Luther: A Blood Diluting Pipette, for Micro-Folin-Wu Method of Blood-Sugar Estimation, Using 0.1 c.c. of Blood, Preliminary Report, Jour. Am. Med. Assn., Aug. 16, 1924, lxxxiii, 508.

1(b) Byrd, T. Luther: Pipeta Para Diluir La Sangre En El Micrometodo De Folin-Wu Para El Calculo Del Azucar Sangueno Utilizando 0.1 c.c. De Sangre, Informe Preliminar, (Spanish) Jour. Am. Med. Assn., August 15, 1924, xii, 262.

2(a) Byrd, T. L.: A Micro-Folin-Wu Method of Quantitative Blood-Sugar Estimation, Using 0.1 c.c. of Blood, Wisconsin State Med. Jour., Feb. 15, 1925.

2(b) Byrd, T. L.: A Micro-Folin-Wu Method of Quantitative Blood-Sugar Estimation, Using 0.1 c.c. of Blood. Jour. of Lab. and Clin. Med., October, 1925, xi, No. 1.

OXALIC ACID, A GOOD WHITE CELL DILUTING FLUID*

By Rodney Jones, Denver, Colo.

ACETIC acid, the standard white-cell diluting fluid, while very good for a quantitative count, is not so good if a differentiation of the polymorphonuclear and the mononuclear cells is desired. It has too great a tendency to destroy the cell membrane and not leave a sharp differentiation between the nuclear and cytoplasmic substance.

After a series of experiments, in which the standard solution of acetic acid was compared with solutions of oxalic acid varying in strength from ½ to 3 per cent, a 2 per cent solution of oxalic acid was found to be the best.

Oxalic acid in my experience comes much nearer being the ideal white-cell diluting fluid. When used in a 2 per cent solution the red corpuscles are sufficiently destroyed, although not as completely as with the acetic acid. The white cells, due to the fact that neither the cell membrane nor the nucleus appear to be destroyed or distorted by the oxalic acid, stand out very clearly. Under low power a much more accurate determination of the percentage of polymorphonuclear cells as differentiated from the mononuclear cells can be made than when the acetic acid solution is used.

When the cells are examined under high power they appear very clear-cut. The cell membrane appears as a light, rather homogenous, but nevertheless distinct shadow, while the nucleus is dark, regular, and in the case of the polymorphonuclear cells even the fine filament extending between the lobes of the nucleus can be demonstrated in the vast majority of cases.

*From the Department of Clinical Pathology, University of Colorado School of Medicine, Denver, Colorado.

Received for publication, October 2, 1926.

DEPARTMENT OF REVIEWS AND ABSTRACTS

ROBERT A. KILDUFFE, M.D., ABSTRACT EDITOR

Clinical and Experimental

CEREBROSPINAL FLUID: Value of Routine Examination of Cerebrospinal Fluid, Crawford, B. L., and Cantrow, A. Am. Jour. Med. Sc., June, 1926, clxxi, No. 6, p. 859.

Report of studies made in 210 cases.

The total amount of spinal fluid is approximately 120 c.c.—completely replaced every five to six hours. Normally there is a constant balance between production and absorption.

Acidosis, anoxemia, anesthesia, and the intravenous administration of extracts of choroid plexus, disintegrating brain tissue, and paretic fluids cause increased flow.

The colloidal-benzoin reaction adds nothing of value to the routine examination.

To prevent disturbance of sugar content by glycolysis, specimens which cannot be examined at once should be placed in sterile tubes containing a few milligrams of sodium fluoride. They can be kept as long as a week at room temperature without marked change.

Normal variations of spinal fluid sugar occur with normal variations of blood sugar maintaining an approximate proportion of 1:2.

For protein content the authors use the following method: To 1 c.c. of spinal fluid add 7 c.c. of distilled water, 1 c.c. of 10 per cent sodium tungstate, and 1 c.c. of $\frac{2}{3}$ N. sulphuric acid. Normal fluids give no precipitate.

In sixty-seven normal cases the cells were four or less per cu. mm.; globulin not increased, and sugar 40-66 mg. per 100 c.c. determined by Foster's modification of the Folin-Wu technic and using a standard containing 5 mg. glucose per 100 c.c.

In eleven children the sugar content was from 71 to 90 mg. with an average of 79 mg.

The essential cause of hyperglychorachia is increased permeability of the protective barrier of choroidal epithelium and cerebrospinal capillary endothelium, seen usually in conditions having an essential vascular pathology such as encephalitis and cerebrospinal syphilis.

Great increase in globulin and sugar content are characteristic of increased intracranial pressure. High sugar values were also found in various functional mental disorders.

The essential cause of low sugar values is glycolysis. It is marked in suppurative and slight in tuberculous meningitis.

The greatest value of sugar determinations is in the differentiation of tuberculous meningitis, from, especially, encephalitis epidemica. Sugar determinations should be a part of every routine examination.

PREGNANCY: The Early Diagnosis of Pregnancy by Precision: Further Observations of Sugar Tolerance Tests. Final Report, Hirst, J. C., 2nd, and Long, C. F. Am. Jour. Med. Sc., June, 1926, clxxi, No. 6, p. 846.

The following methods were reviewed:

1. The Abderhalden reaction; concluded to be of no value because of the large number of nonspecific reactions.

2. Erede's anaphylactic reaction: without experimental substantiation.

3. Casta's novocaine-formalin reaction: numerous nonspecific reactions with normal blood and in infections and toxic conditions.

4. Dienst's reaction: valueless even in pregnancy.

5. Sedimentation test: worthless for the diagnosis of pregnancy though of interest in other conditions.

6. The alimentary glycosuria test of Frank and Nothmann. A useful test with a margin of 6 to 8 per cent error.

The method used is as follows:

A. Average supper given the night before.

B. Collect first morning specimen of urine which must be negative to Fehling's solution.

C. Omit breakfast.

D. Give 7.5 gm. of table sugar per 10 pounds of body weight except that the maximum amount must not be over 150 gm. This is dissolved in two glasses of water each containing the juice of half a lemon.

E. Voided specimens are collected at one and two hours after the sugar ingestion and qualitatively tested for sugar by Fehling's method.

If either of the hourly specimens shows a definite reaction for sugar the test is positive. "Traces" or "slight reductions" are disregarded. The reactions must be frankly positive or negative.

Precautions must be taken to see that the entire dose of sugar is taken and that none is lost by vomiting. Patients intolerant to sugar because of various conditions are not amenable to the test.

7. The Raubitschek adrenalin test: inconstant and untrustworthy.

8. The Phlorizin test: prone to false positive results and untrustworthy.

DIABETES: Relation of Abdominal and Rectal Infections to the Pathogenesis of Diabetes Mellitus, Visher, J. W. Am. Jour. Med. Sc., June, 1926, clxxi, No. 6, p. 836.

Case reports tending to support the following premises:

The underlying pathologic change in diabetes in many cases is a pancreatitis.

The pancreatitis may originate in acute infectious diseases, and from hematogenous focal infections.

Five cases of diabetes mellitus apparently secondary to abdominal and rectal infections are reported, with improvement following surgical intervention.

The opinion is ventured that in these cases the infection reached the pancreas through the lymphatics, either directly or by way of the portal circulation.

The conclusion is suggested that abdominal and rectal infections are important etiologic factors in the etiology of diabetes mellitus.

CARCINOMA: The Bacterial Flora of Cancer of the Breast, Warren, S. L. Am. Jour. Med. Sc., June, 1926, clxxi, No. 6, p. 813.

Micrococci and diphtheroids have been cultured readily from cancer tissue obtained from seven human breasts, without obvious areas of infection. They also were present in a breast with chronic mastitis and in parts of a breast not involved by cancer.

It would appear that these organisms are casual inhabitants of the breast structures, and play no direct part in the production of cancer of the breast.

The literature bears witness to the fact that Nuzum's micrococcus, which seems the same as the one described here, has been obtained at different times in the past though given various names.

CARCINOMA: The Repeated Inoculations of Animals with So-Called "Cancer Organisms," Warren, S. L., and Pearse, H. E. Am. Jour. Med. Sc., June, 1926, No. 6, p. 820.

Two hundred and forty-one mice of a strain susceptible to mouse cancer inoculations, but in which spontaneous tumors were very rare, were given at weekly intervals intracutaneous injections of either the micrococcus of Nuzum or diphtheroids and micrococci obtained from human breast cancers. The inoculations were continued until death, or for four months, at which time only fifty mice remained alive, the others having died, usually from septicemia. These fifty mice were observed for two months more, or for a total of six months.

Ulcerations of the skin which readily healed occurred with great regularity. None of the animals showed any evidence of a neoplastic growth except one which developed a spontaneous tumor of the liver.

Four rabbits receiving weekly injections of both diphtheroids and micrococci for three to five months, showed no signs of malignant disease even at the end of six months.

No evidence was found that any of these organisms play a primary rôle in the etiology of cancer, though an indirect rôle is possible.

PREGNANCY: Interagglutination of Maternal and Fetal Blood in the Late Toxemias of Pregnancy, Allen, W. M. Bull. Johns Hopkins Hosp., March, 1926, xxxviii, No. 3, p. 217.

A study concerned with the possible relation of blood incompatibility as a cause of eclampsia, in which the isoagglutination relationship of 479 mothers and their infants was investigated.

Microscopic technic was used routinely.

The cases were divided into six groups: (1) normal; (2) questionable; (3) preeclamptic toxemia; (4) eclampsia; (5) nephritic toxemia without convulsions; (6) nephritic toxemia with convulsions.

There is no evidence that incompatibility is more frequent in toxemic than in normal gestation. Incompatibility between the bloods of mother and infant was present in 20.8 per cent of 375 normal and 21.1 per cent of 104 toxemic pregnancies.

There is no evidence of an increased agglutinin titer in the maternal serum of toxemic women.

There is no evidence of specific immunization of the mother against fetal corpuscles.

The discrepancy between this and previous work is probably accounted for by the size of the series studied. With a small number of cases the percentage of error and likelihood of coincidence are very great.

The study of this series of cases, by the methods used, gives no evidence that the late toxemias of pregnancy have their origin in isoagglutination phenomena.

TUBERCULIN TESTS: Intracutaneous Tests with Human and Bovine Tuberculin, Downing, H. F., and Higgins, H. L. Am. Jour. Dis. Child., February, 1926, xxxi, 178.

From a study of fifty-one cases the following conclusions are formulated:

Both bovine and human tuberculin should be employed in routine tuberculin tests, not only to determine the diagnosis of the type of tubercle bacilli present but also to increase the value of the test by detecting cases which would otherwise be missed. In our series, the missed cases would have amounted to 15 per cent of the total tuberculous patients.

Evidence from two cases tend to confirm earlier reports that in the first stages of tuberculosis, the patient will react to but one type of tuberculin, presumably that of the infecting type, while later the patient reacts to other types (group reaction).

UREMIA: On the Presence of Cyanate in the Blood, Gottlieb, E. Brit. Biochem. Jour., 1926, xx, 1.

In view of the fact that it has been asserted that considerable quantities of cyanate are found in the blood and, also, that cyanic acid may give rise to uremia Gottlieb reports his experiments on this matter.

Cyanate is rare in the blood in concentrations over 0.1 mg. per 100 c.c. of plasma.

Cyanate per os or intravenously is toxic; hence cyanate as present in ordinary solutions cannot be considered as a precursor of uremia.

MERCUROCHROME: Unsuccessful Experiments with Mercurochrome as a Biliary Antiseptic. IX. Experimental Typhoid-Paratyphoid Carriers, Meyer, K. F., Sommer, H., and Eddie, B. Jour. Infect. Dis., June, 1926, xxxviii, No. 6, p. 469.

Mercurochrome intravenously injected is excreted in the hepatic bile of rabbits in concentrations which may destroy 10,000,000 typhoid bacilli in from six to twenty-four hours. It has been impossible, however, to cure experimentally produced gall bladder carriers by giving mercurochrome intravenously or by mouth.

The colorimetric methods used in the estimation of mercurochrome, the influence of proteins, etc., on the germicidal properties of the compound are discussed.

Laboratory Technic

BLOOD SEDIMENTATION: The Graphic Presentation of the Blood Sedimentation Test. A Study in Pulmonary Tuberculosis, Cutler, J. Am. Jour. Med. Sc., June, 1926, clxxi, No. 6, p. 882.

Reviewing the variety of methods which have been and are employed in this procedure and which render difficult an accurate comparison of results, Cutler presents a simple method and a graphic report utilized by him in a study of pulmonary tuberculosis.

A 5 c.c. tube, graduated in tenths of a cubic millimeter, each 1 mm. in height, and marked in millimeters is required. This tube may be obtained from the A. H. Thomas Co., Philadelphia, Pa.

Blood is aspirated to the 5 c.c. mark in a syringe containing 0.5 c.c. of 3 per cent sodium citrate (freshly made), mixed by tilting the syringe, and, after removal of the needle, emptied into the tube. Several specimens may be taken at once and the tubes marked.

On arrival in the laboratory, the tubes are stoppered with paraffined corks and gently inverted several times.

They are replaced in the rack and readings made every five minutes for one hour, the readings being graphed on a special chart. Readings may be made any time up to ten hours after collection of the blood. On these charts, obtainable from C. M. Beckemeyer, Sellersville, Pa., the horizontal lines represent the divisions of the sedimentation tube, the vertical lines intervals of time. A graph is thus constructed.

The author uses the term "sedimentation index" to express the total sedimentation of the blood cells at one hour.

The normal index for men is from 2 to 8; for healthy women from 2 to 10; during menstruation as high as 12 mm.

By "sedimentation time" is meant the period elapsing before packing of the red cells sets in. Normally this is always a question of hours.

The greater the sedimentation index and the shorter the sedimentation time, the greater is the pathologic activity and vice versa.

In healthy individuals the graph is always a straight line.

Cutler regards the test as a nonspecific reaction occurring in many diseases but also looks upon it as a valuable aid in estimating the activity of tuberculous lesions.

He emphasizes the value of the graphic method described.

The paper is illustrated with six figures including a reproduction of the chart.

BLOOD SUGAR: A New Titrimetric Principle and Its Application to the Determination of Uric Acid and Blood Sugar, Flatow, L. München. med. Wchnschr., November 20, 1925, lxxii, 2009.

Proteins are removed with 10 per cent sodium tungstate and two-thirds normal sulphuric acid.

The following reagents are required:

1. Potassium ferricyanide 0.0803 gm., distilled water 100 c.c. Keep in a dark bottle. The solution is stable. 1 c.c. = 0.1 gm. of dextrose.

2. Sodium indigomonosulphate 0.3 gm. in 1000 c.c. of distilled water.

3. A 15 per cent cold neutralized sodium carbonate solution and its tenfold dilution.

Method: 0.1 c.c. of blood is taken from the finger with a standardized pipette and transferred to a centrifuge tube containing 1.7 c.c. of distilled water. Add 0.1 c.c. of tungstate solution and mix, add 0.1 c.c. of sulphuric acid and shake well.

Centrifuge and transfer 1 c.c. to a wide test tube, (or 0.5 c.c. plus 0.5 c.c. of distilled water) add 2 c.c. of ferricyanide solution and 0.5 c.c. of 1.5 per cent sodium carbonate solution.

In a second tube, (blank test), place 1 c.c. of water, 2 c.c. of ferricyanide solution and 0.5 c.c. of 1.5 per cent sodium carbonate solution.

Place both tubes in a water-bath and boil fifteen minutes. Cool and titrate with the indigomonosulphate solution until the first bluish color is constant for 1 minute. A few drops of the 15 per cent sodium carbonate solution are added before the titration.

The amount of indigo required for titration in the sugar test, (b), is subtracted from the amount used in the blank test, (a), and the result divided by (a) and multiplied by 0.2 equals the sugar content.

$$\text{Sugar} = \frac{(\ a - b\ )}{(\ a\ )} \times 0.2$$

The method as applied to the determination of uric acid is indirect and cumbersome.

IMMUNITY: The Isolation of Substances with Immune Properties, Locke, A., and Hirsch, E. F. Jour. Infect. Dis., November, 1925, xxxvii, 449.

A method is presented for the preparation of a highly purified hemolysin by selective adsorption and a destruction of the combining capacity of the homologous erythrocyte by ether extraction.

The procedure permits the securing of hemolysin of such purity that but 0.000,125 to 0.000,18 mg. of protein are associated with each hemolytic unit.

Fresh sheep blood is defibrinated, centrifugated, and the cell sediment washed five times with 0.9 per cent sodium chloride solution. Fifteen c.c. of the packed, washed cell sediment are equally divided, between two large centrifuge tubes, and to each portion there is added quickly and with vigorous shaking, 40 c.c. of perfectly fresh rabbit antisheep serum. After two hours, the cells have completely laked and the stroma has flocculated and settled toward the bottom of the tubes. Centrifugation for forty-five minutes completes the separation; 90 to 99 per cent of the hemolysin originally present is found to be bound to the stroma sediment. The brilliantly clear, red supernatant liquid is decanted from the sediment and replaced by an equal volume of 0.9 per cent sodium chloride solution. After the stroma is finely suspended in the wash liquor by prolonged shaking, the suspension is allowed to stand for twenty minutes and is then strongly centrifugated for thirty minutes. The washing process is repeated (about six times) until the supernatant liquid has no trace of color and gives no trace of foam when shaken. The stroma obtained is perfectly white and has lost little of the originally bound hemolysin.

The well washed, hemolysin-saturated stroma is extracted with ether three times. The volume of the stroma decreases 90 per cent under this treatment. After the removal of the third ether extract, the ether remaining dissolved in the stroma material is removed by centrifugation in a warm centrifuge. The stroma material packs at the bottom of the tube and the salt solution, which made up the cell volume, may be decanted. The residue is washed twice with 0.9 per cent salt solution and once with distilled water. Considerable hemolysin is lost to the salt solution, but protein impurities, due to surface adsorption, are thereby almost completely removed. The hemolysin of the salt extracts may be recovered by electrodialysis and isoelectric flocculation. The washed residue is extracted repeatedly with N/1000 sulphuric acid, the extracts are pooled, flocculated by neutralization, and the suspension centrifugated. The flocculation is quantitative, as hemolysin is almost insoluble in pure water. The precipitated material is extracted with 0.9 per cent salt solution, and solutions of any desired hemolytic content may be obtained by varying the amount by salt solution used. The hemolytic unit of the extract is associated with from 0.000,125 to 0.000,18 mg. of protein. The preparations still contain a small amount of cell globulin but are probably 30 per cent or more pure hemolysin. Their combining power for erythrocytes far exceeds that of ordinary immune serums, indicating that their content of stroma material is very small. The amount of the original hemolysin which is recovered in these preparations is 30 to 50 per cent.

BLOOD, IRON IN: Determination of Iron in Blood, Tissues, and Urine, Fowweather, F. S. Brit. Biochem. Jour., 1926, xx, 93.

Method of determination of iron in blood.—One c.c. of blood is measured into a test tube containing 4 c.c. of distilled water. After thorough mixing, 1 c.c. of this diluted blood is transferred to a Pyrex test tube (200 x 25 mm.) followed by 1 c.c. of concentrated sulphuric acid. The tube is clamped and held at an angle of 40° to the horizontal. The contents are boiled rather vigorously until practically all the water has been driven off and white fumes begin to be evolved. Heating is discontinued for about half a minute, after which time 0.5 c.c. of "perhydrol" is added to the tube, drop by drop, from a teat pipette. Boiling is then repeated. A brisk evolution of oxygen occurs and the solution in the tube assumes an amber color. When white fumes are again evolved, heating is again discontinued and after cooling for half to one minute a further 0.5 c.c. of "perhydrol" is added as before. Heating is resumed and the solution usually becomes colorless. If all the color has not disappeared an additional 0.3 c.c. of "perhydrol" is added. Heating is continued for one minute after the solution has become colorless. The solution is now allowed to cool completely, then it is diluted with about 5 c.c. of distilled water and transferred to a 50 c.c. stoppered graduated flask. Into another similar flask are placed 1 c.c. of a standard iron solution containing 0.1 mg. iron per c.c. and 1 c.c. concentrated sulphuric acid. Water is added to both flasks up to a volume of about 18 c.c. after which 25 c.c. acetone are added. The contents of the flasks are thoroughly mixed and allowed to cool at room temperature. To each flask are then added 5 c.c. of 3 M ammonium thiocyanate, the contents mixed and made up to the mark. The two solutions are then compared in a colorimeter.

If the standard is set at 20 mm., then

$$\frac{20}{R} \times 50 = \text{mg. iron per 100 c.c. blood,}$$

or $\frac{20 \times 50}{R \times 3.35}$ hemoglobin in blood (hemoglobin contains 0.335 per cent of iron), where R is the colorimeter reading of the solution tested.

Preparation of standard iron solution: Dissolve 0.7 gm. pure ferrous ammonium sulphate in about 50 c.c. of distilled water. Add to the solution 20 c.c. 10 per cent iron-free sulphuric acid, warm slightly and then add 0.1 N (approx.) potassium permanganate solution to oxidize the ferrous salt completely. Dilute with distilled water to 1l. Each c.c. contains 0.1 mg. iron.

Method of determination of iron in tissues.—The organ or tissue to be examined is minced and washed with water to remove blood. It is then dried in a steam oven. The dried material is then ground in a mortar until it passes through a 30 mesh sieve.

One gm. of this material is weighed into a 300 c.c. Kjeldahl flask to which are added 10 c.c. concentrated H_2SO_4. The flask is heated in a fume chamber, gently for fifteen minutes, and then strongly for thirty minutes. At the end of this time the walls of the flask are free from charred material and its contents consist of a brown homogeneous fluid. This is allowed to cool and then diluted with an equal volume of water and transferred to a 50 c.c. stoppered graduated flask. A rise of temperature occurs on diluting and washing the fluid into the graduated flask. When room temperature is again reached the contents of the flask are made up to the mark. A certain amount of light, flocculent precipitate forms on adding water to the acid digestion fluid. The contents of the flask are therefore shaken and 5 c.c. are withdrawn immediately and placed in a Pyrex tube, to which 1 c.c. of perhydrol is added. The tube is inclined as in the previous method and the oxidation with perhydrol there outlined is followed. If much iron is present the final solution has a slight yellow color after oxidation which almost entirely disappears on cooling.

After cooling, the solution is transferred to a 50 c.c. graduated flask and acetone and thiocyanate solution added as before, a standard for comparison being also prepared as already described.

In the case of organs containing more than the normal amount of iron (where siderosis is present) 0.5 gm. should be taken instead of 1 gm., and it may be found necessary to prepare a stronger standard solution for colorimetric comparison.

Method of determination of iron in urine.—To 100 c.c. of the twenty-four-hour specimen of urine placed in a 300 c.c. Kjeldahl flask, 10 c.c. of concentrated sulphuric acid are added. The contents of the flask are vigorously boiled until frothing begins to take place. Heating is then carried on with caution until frothing ceases. A full flame is then used again until the walls of the flask are free from charred material and homogeneous fluid results. After cooling, this fluid is diluted and transferred to a 50 c.c. graduated flask whose contents are made up to the mark when they have cooled again to room temperature. Ten c.c. of the liquid are transferred to a Pyrex tube and concentrated by boiling. Until the volume has been considerably reduced the flame of the burner must not be directed to the bottom of the tube but to the wall of the tube just below the surface of the liquid. When nearly all the water has been driven off and white fumes begin to be evolved 0.5 c.c. perhydrol is added as before, followed by further quantities of 0.3 c.c. and 0.2 c.c. After the last addition the resulting fluid is boiled vigorously for three minutes, when practically no more fumes are evolved. The liquid is then allowed to cool, diluted with about 2 c.c. distilled water and transferred to a 25 c.c. stoppered graduated flask. One c.c. concentrated sulphuric acid is added and 12.5 c.c. acetone. The contents of the flask are made up to about 20 c.c. and allowed to cool to room temperature. Three c.c. of the thiocyanate solution are then added and the contents of the flask well mixed and made up to the mark. The standard for comparison is prepared at the same time in a similar flask using 1 c.c. of a solution containing 0.01 mg. iron per c.c., 1 c.c. concentrated sulphuric acid, and 12.5 c.c. acetone. The unknown solution as thus prepared is cloudy. A portion of it is therefore immediately centrifuged in a closed tube for five minutes, when the clear supernatant fluid which results is compared with the standard in the colorimeter.

The iron solution used for preparing the standard for comparison is one-tenth the concentration of the standard previously used and is prepared from the latter by suitable dilution with distilled water.

It is essential in this method, where very small quantities of iron are estimated, to prevent dust from falling into the solutions, as it has been found that the atmospheric dust, at any rate in an industrial center, contains sufficient iron to cause errors in the result if precautions are not taken to exclude it.

REVIEWS

Books for Review should be sent to Dr. Warren T. Vaughan, Medical Arts Building, Richmond, Va.

*Principles and Practice of Chemotherapy**

UNTIL comparatively recent times, the therapeusis of disease has been mainly symptomatic and, to a greater or lesser extent, empiric; modern developments in knowledge of the parasitic causes of disease and the consequent better understanding of the mechanism concerned in the production of the pathology associated with the disease resulting, have led to a definite and systematic search for specific remedies or methods of treatment to which attempts the term chemotherapy is applied.

Originating largely in the work of Ehrlich which had its inception in the thought "that the ways and means by which drugs are distributed over the body must be of the greatest importance in the rational development of therapeutics," modern chemotherapy is defined by Kolmer as: "The prevention or treatment of disease by chemical disinfection or inhibition of the parasitic causes without marked or serious toxic effects" upon their host.

But little consideration of this definition, and but a casual survey of the literature concerned with it during the last decade alone, suffices to bring a realization of the broad field involved and the numerous, complex, and interrelated problems presented for study and consideration.

Until the publication of Dr. Kolmer's book, there existed nowhere in any language a systematic or comprehensive survey of this complicated subject.

Had Dr. Kolmer done nothing more than present a survey of the literature of modern chemotherapeutic studies he would have rendered an invaluable service to all who are concerned with the prevention and treatment of disease.

He has done more than this, however. Not only are the literature and studies of the world surveyed and presented in a systematic manner, but the results are reviewed and judged in the light of the comprehensive experience of a worker intimately concerned with the development of the subject.

The book is divided into ten parts, all interlocking and welded into a harmonious whole.

After an historic discussion, Part I considers in detail the methods for determining the toxicity of chemical agents in relation to their chemotherapeutic use. Part II is a comprehensive discussion of the chemotherapy of bacterial and mycotic disease in man and the lower animals. Part III discusses the chemotherapy of trypanosomal diseases. Part IV discusses the chemotherapy of spirochetal diseases other than syphilis. Part V takes up the chemotherapy of protozoan and metozoan diseases excluding trypanosomal and spirochetal diseases. Part VI deals with the chemotherapy of diseases of unknown or doubtful etiology.

These sections cover 446 pages and bring to the reader a well-planned, clearly presented, and eminently practical discussion of the entire field of modern chemotherapy.

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*Principles and Practice of Chemotherapy, with Special Reference to the Treatment of Syphilis. By John A. Kolmer, Professor of Pathology, Graduate School of Medicine, University of Pennsylvania. Pp. 1106. 82 illustrations. Cloth. Price $12.00 net. W. B. Saunders Co., Philadelphia.

NOTE: In so far as practicable the book review section will present to the reader (a) interesting knowledge on the subject under discussion, culled from the volume reviewed, and (b) description of the contents so that the reader may judge as to his personal need for the volume.

We trust that the scientific information printed in these pages will make the reading thereof desirable per se and will thereby justify the space allotted thereto.

There is no specialty of medicine or surgery to which this portion of the book is not applicable; no worker who cannot find information and assistance applicable to his particular problem.

What has been done, how to do it, what might be done, and practical suggestions for its accomplishment abound on every page. Nothing is left to guesswork; everything is detailed. The physician, the surgeon and the laboratory worker may all profit by the material here presented.

Part VII and the remainder of the book (659 pages), are devoted to the treatment of syphilis.

There is much to support the contention that, generally speaking, syphilis is a very much mishandled disease. There are too many for whom the study and diagnosis of syphilis begins and ends with the Wassermann test; too many whose treatment is of the disease rather than the patient with the disease; too many whose scheme of treatment is that furnished by the detail man or the manufacturer of preparations for the purpose.

There are too many so-called serologists, also, whose knowledge is entirely technical and who fail to realize or are ignorant of the relation of the serology of syphilis to its pathology and to the variations related to its pathology and treatment.

Standard or set methods for the treatment of syphilis are impossible to formulate. The successful treatment or even an intelligent attempt involves an appreciation and understanding of all the minutiae which affect the particular case in point.

It is surely not too much to expect of any one undertaking the treatment of syphilis that he shall have some intelligent conception of the rationale underlying the various drugs available for the purpose; some understanding of what they are, how they act, when they may and may not be used, and something concerning the nature and prevention of untoward by effects.

All this is discussed in great detail as related to all the preparations utilized in modern syphilology. The discussion of various types of reactions is a mine of information on the subject and summarizes all that can be said at present concerning their prevention or treatment and, as far as the reviewer knows, this information has not before been presented in so detailed and practical a manner in any one book.

While the methods used by Kolmer for the treatment of syphilis are presented in detail, the necessity for the individualization of treatment is consistently emphasized and all the information available for an understanding of the subject is presented and discussed.

While of great value to the syphilographer and serologist, this section of the book should prove of inestimable value to the practitioner at large for the comprehensive information it contains relative to the therapeutic value of the many methods and preparations now in use; for its clear and thorough discussions of the serology of the disease and its clinical application; the causes, prevention, and treatment of arsenical and other reactions; and especially the detailed presentation of methods.

All in all, the book is a worthy companion and supplement to the author's previous volume on "Infection, Immunity, and Biologic Therapy" and deserves a place in the library of all who are concerned with the study, treatment, or prevention of disease.

The first of its kind in any language it is doubtful if this book will ever be displaced from the commanding position it now occupies with reference to its subject.

The Journal of Laboratory and Clinical Medicine

VOL. XII ST. LOUIS, MO., MARCH, 1927 NO. 6

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Official Organ of the American Society of Clinical Pathologists

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EDITORIALS

Ephedrine

IT HAS been a long time since the question was first asked, "Can any good come out of Nazareth?" In a somewhat similar spirit the question might have been asked only a few years ago, Can any good come out of Chinese medicine? Whether by chance or by actual scientific trial we cannot say, nevertheless we can now answer the latter question with a positive yes, for from out of the superstition, the medical riff-raff and the folklore of more than five thousand years a new drug—for us—ephedrine has emerged. As early as 1885 and 1887 this drug (or an alkaloid supposed to be ephedrine) had been isolated by Yamanashi and Nagai from ephedra vulgaris, var. helvetica. The popular name of this rather common Chinese plant is Ma Huang and other alkaloids are also present in it. The earlier pharmacologic work on ephedrine was somewhat confusing and the investigations were, unfortunately, not followed up as they should have been.

Structurally the drug is rather similar to epinephrine and tyramine and like them it possesses sympathomimetic properties. Beginning with Amatsu and Kubota in 1917 and Chen and Schmidt about 1923 a rapidly growing list of pharmacologists and clinicians have carried out a brilliant and very promising series of experiments on this drug. Already an important future for this alkaloid is definitely indicated. It is practically certain to displace the use of epinephrine in certain types of cases. And in all probability it will find a use in new fields of its own. The pharmaceutical drug manufacturers have already begun to read the signs of the future with reference to this compound and it appears that various manufacturers in this country have now laid in stock, or are trying to get, a goodly supply of the crude Ma Huang. These companies have commendably proceeded with caution with reference to the new drug. For notwithstanding the many interesting and valuable points which have already been discovered about this substance, it appears that more is yet to follow.

Briefly stated the drug has been shown to raise the blood pressure, cause dilatation of the pupil, contract the uterus, relax the intestinal and bronchial musculature, increase the blood sugar, stimulate the heart under some conditions and apparently depress it under others, to stimulate the central nervous system in some degree, to cause death by heart failure and to possess a relatively low toxicity. The rise in blood pressure produced by ephedrine is neither so abrupt nor so high, but lasts very much longer than that produced by epinephrine. And ephedrine solutions are very stable, can be sterilized by boiling and the drug can be effectively administered by stomach as well as hypodermically. It appears that neither animals nor man develop any special tolerance for the drug when it is administered in repeated doses from day to day over comparatively long periods. But on intravenous injection it is found that the first dose, if it be large enough will produce a maximum rise of pressure, the second dose (of the same amount) will then raise the pressure only one-half or two-thirds as high as the first and following doses, if given within ten or fifteen minutes of each other, tend progressively to produce less and less of a rise in arterial pressure, and usually the later doses may produce some temporary fall in pressure. In a number of recent experiments the writer has found that when this stage has been reached, and ephedrine in any sized dose will no longer produce a rise in pressure, then an injection of epinephrine will promptly raise the pressure almost as high as the given dose would have raised it before any ephedrine had been given. This observation can only mean that ephedrine and epinephrine do not act on identically the same structures, or else that the two drugs do not affect them in the same identical way. Many years ago Barger and Dale investigated the pharmacologic action of a large number of compounds to which they gave the name of "sympathomimetic amines." These authors showed that the relative action of the various members of the series on diverse organs and structures innervated by the true sympathetic nerves varies considerably, and that a member which was very active on one organ might not manifest this same degree of activity on another presumably similarly innervated structure or organ. It is to be ex-

pected that in due course this same observation will be made in various directions with reference to ephedrine also. And in this connection it may be noted that the ancient Chinese used Ma Huang as a diaphoretic (possibly without justification) and recent pharmacologic work has shown that ephedrine stimulates the stellate ganglia. There is some stimulation of the sympathetic nerve supply to the secretory glands and this also seems to depart in some degree from the typical epinephrine action. Apparently the central nervous stimulation may occasionally cause sweating in the case of toxic effects from very large doses. The unequal contractions of the various parts of the systemic vasculature would seem to produce temporarily a somewhat different distribution of blood in the various organs and parts of the body from that which follows the administration of epinephrine. Synergism has been noted between ephedrine and epinephrine and between ephedrine and tyramine.

After doses of ergotamine sufficient to produce a fall in blood pressure when epinephrine was injected Nagel produced a rise in pressure by injection of ephedrine.

The minimal lethal dose intravenously for dogs was found by Chen to be from 70 to 75 mg. per kilogram of weight. From this it might be inferred that a man of 150 pounds weight would be fatally poisoned by some 60 to 70 grains if injected intravenously. In contrast to this the usual clinical dose is from 1 to $2\frac{1}{2}$ gr. and $6\frac{2}{3}$ gr. have been given in a single dose without untoward effects.

From a clinical standpoint the best results have so far been obtained when the drug was applied locally to the nose in cases of chronic congestive conditions such as hypertrophic rhinitis and hay fever. In asthma it has also been found of definite value, and in the treatment of urticaria and anaphylaxis the drug would appear to have a promising future. A rather unexpected feature of the action of ephedrin was the observation that it appeared to be the best single drug to use as a respiratory stimulant in cases of narcotic poisoning. And the drug has also a certain field of usefulness as a mydriatic.

The use of the drug as a cardiac or circulatory stimulant is still in the experimental stage and the general results in the field have not so far been as promising as might have been expected. But there is still a possibility that the substance may be found valuable in certain types of cardiac or circulatory conditions. The drug apparently cannot replace epinephrine as a local styptic for use in local anesthetic solutions for its local vasoconstricting action is too slow to prevent the general absorption of the anesthetic.

It is obvious that modern pharmacologists—and significantly enough the foremost of these is Dr. K. K. Chen—are now interpreting the dream of Emperor Shen Nung (3217 B. C.) who *tasted* Ma Huang and then wrote it down in his *Pentsao*, or pharmacopeia, as a good and useful drug. Could his imperial majesty now come back to earth he would be in a position to exclaim with modern inspiration, All things come to him who waits 5000 years!

—*D. E. J.*

The Menace of the Slightly Positive Wassermann

PARADOXICAL as it may seem, the obvious sometimes requires recurring or persistent reiteration before it is generally appreciated.

While the subject is not altogether new, has been discussed by various writers, and commented upon in numerous journals, including this one,[1] a recent paper by Mitchell[2] again utters a timely warning relative to the mal-interpretation of the Wassermann reaction as a potent cause of syphilophobia.

"As a disseminator of false diagnosis of syphilis" says Mitchell, "the slightly positive Wassermann report was without a rival until the advent of the Abrams machine" and the paper recounts illustrations of the harm done by the erroneous interpretation of such reports.

Mitchell believes that there is a moral obligation on the part of serologists to make it clearly understood by clinicians that the Wassermann report is frequently only a symptom and that, in case of doubt, further specimens should be examined.

Let it be said and emphasized that this, indeed *is* the *serologist's* conception of the test and that it is the serologist's further contention that there is a very definite moral obligation upon the part of the clinician to become familiar with this and many other pertinent facts related to the diagnosis and treatment of syphilis—an obligation too often neglected and unrecognized.

It is unfortunate for the patient; a stigma upon the profession at large; a potent factor in the production of syphilophobia; a still more potent factor in sowing a crop of partiallly treated syphilities from whom will be reaped a generous harvest of neurosyphilities in the years to come; and a reproach to the physician in general that the introduction of the Wassermann test and the arsenicals have brought about, as a by-product, a number of pseudosyphilographers for whom, as Breman[3] has said, "The clinical study of syphilis is unnecessary. The public Wassermann laboratory makes the diagnosis . . . . and a few injections of arsphenamine clear up the lesions."

The responsibility for this situation rests more heavily upon the clinician at large than upon the serologist.

Be it said to the serologist's credit that he has always emphasized the status of the Wassermann reaction as but one phase in the examination of the patient for evidences of syphilis; that he has contributed greatly to an understanding of its limitations; reiterated again and again the vital necessity for its clinical interpretation and correlation with all the other features of the particular case; and pleaded vehemently for some clinical interest and understanding of its mechanism and clinical significance.

There are, it is true, unscrupulous and mercenary serologists just as there are unscrupulous and ignorant clinicians and both should be sought out and scourged from the profession.

The subject has been discussed and rediscussed and, nevertheless is still worthy of attention.

This much may be again said with confidence:

As long as there are physicians for whom a Wassermann test is a Wassermann test regardless of how or by whom performed; as long as the physician selects his serologist without careful consideration of his ability, technical skill, and professional standing; as long as physicians vary in the thoroughness of their knowledge and understanding of syphilis in general and especially its serology; as long as any one can open a laboratory, proclaim himself a serologist, and have his reports accepted without question; as long as physicians neglect to choose a serologist who can be, when required, a consultant; and as long as laboratory reports are made to take the place of careful, painstaking, and intelligent studies of each individual case—just so long will errors, fallacies, and clinical misinterpretations leading to syphilophobia and worse evils be perpetuated.

As has been said before in these pages[2] "The acceptance of a Wassermann report at its 'face value' indicates a lack of understanding of the factors influencing the occurrence and detection of the reaction, and an equally serious lack of appreciation of the essential necessity for a careful study of each case upon its individual merits."

REFERENCES

[1]Kilduffe, R. A.: The "Face Value" of the Wassermann Report, JOUR. LAB. AND CLIN. MED., November, 1925, xi, 2.
[2]Mitchell, J. H.: The Menace of the Slightly Positive Wassermann Reaction, Jour. Am. Med. Assn., October 23, 1926, lxxxvii, 1351.
[3]Breman, C. J.: A Thorough History An Important Factor in Syphilis, Am. Jour. Syph., 1921, v, 565.

—*R. A. K.*

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